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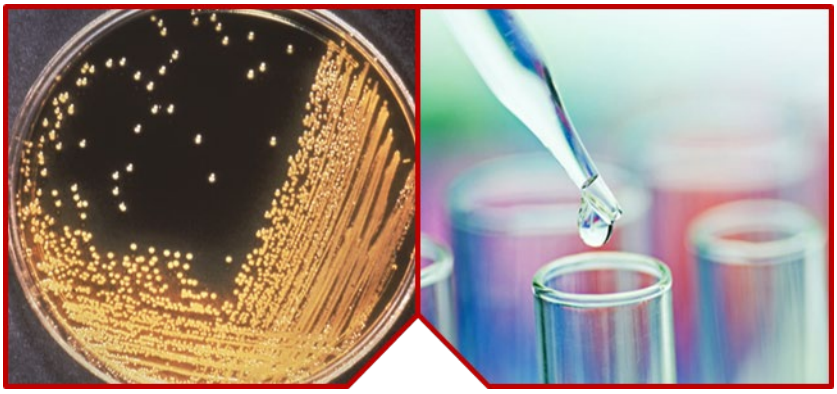
Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2022



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Office of Research and Development
Homeland Security Research Program

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Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2022

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Cincinnati, OH 45268

Office of Research and Development
Homeland Security Research Program

Disclaimer

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development (ORD) conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation's environmental challenges. The Center's research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and ground water, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

The purpose of Selected Analytical Methods for Environmental Remediation and Recovery (SAM) is to identify the analytical methods that will be used in cases when multiple laboratories are called on to analyze environmental samples in support of EPA remediation and recovery efforts following an intentional or accidental homeland security-related contamination incident. The information is intended for use by EPA and EPA-contracted and -subcontracted laboratories, such as the Environmental Response Laboratory Network (ERLN) and Water Laboratory Alliance (WLA). It can also be used by other agencies and laboratory networks and as a tool to assist state and local laboratories in planning for and analyzing chemical, biological and/or radiological (CBR) environmental samples and radioactively contaminated outdoor building material samples.

Gregory Sayles, Director
Center for Environmental Solutions and Emergency Response

Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2022

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Abbreviations and Acronyms

A-230	Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate
A-232	Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate
A-234	Ethyl N-[(1E)-1-(diethylamino)ethylidene]-phosphoramidofluoridate
ACS	American Chemical Society
AOAC	AOAC International (formerly the Association of Official Analytical Chemists)
APCI	Atmospheric Pressure Chemical Ionization
APHA	American Public Health Association
APHL	Association of Public Health Laboratories
ASM	American Society for Microbiology
ASR	Analytical Service Requests
ASTM	ASTM International (formerly the American Society for Testing and Materials)
ATP	Alternate test procedure
ATSDR	Agency of Toxic Substances & Disease Registry
AWWA	American Water Works Association
BA	*Bacillus anthracis*
BAM	Bacteriological Analytical Manual
BCYE	Buffered charcoal yeast extract
BCYE GPCV	Buffered charcoal yeast extract with glycine, polymyxin B, cycloheximide and vancomycin
BCYE PCV	Buffered charcoal yeast extract with polymyxin B, cycloheximide and vancomycin
BEH	Ethylene-bridged hybrid
BGMK	Buffalo green monkey kidney
BHT	Butylated hydroxytoluene
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BoNT	Botulinum neurotoxin
BSL	Biosafety level
BTX	Brevetoxin
BZ	Quinuclidinyl benzilate
°C	Degree Celsius
CAS RN	Chemical Abstracts Service Registry Number
CBR	Chemical, biological and/or radiological
CCD	Charge-coupled device
CCID	Coordinating Center for Infectious Diseases
CDC	Centers for Disease Control and Prevention
CESER	Center for Environmental Solutions and Emergency Response (EPA)
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition (U.S. Food and Drug Administration)
CHCA	α-cyano-4-hydroxycinnamic acid
CLLE	Continuous liquid-liquid extraction
CLP	Contract Laboratory Program
CPE	Cytopathic effect
cps	Counts per second
C_T	Cycle threshold
CVAA	2-Chlorovinylarsonous acid
CVAFS	Cold vapor atomic fluorescence spectrometry
CVAOA	2-Chlorovinylarsonic acid
CWA	Chemical Warfare Agent
2,4-D	2,4-Dichlorophenoxyacetic acid
DA	Domoic acid
DAI	Direct aqueous injection

DAPI	4',6-Diamidino-2-phenylindole
DAS-HG-HSA	Diacetoxyscirpenol hemiglutarate human serum albumin
DAS-HS-HRP	Diacetoxyscirpenol hemisuccinate horseradish peroxidase conjugate
DB-1	100% Dimethylpolysiloxane
DBPR	Division of Bioterrorism Preparedness and Response
dcNEOSTX	Decarbamoylneosaxitoxin
dcSTX	Decarbamoylsaxitoxin
DELFIA	Dissociation-Enhanced Lanthanide Fluorescence Immunoassay
DHHS	U.S. Department of Health and Human Services
DHS	U.S. Department of Homeland Security
DIC	Differential interference contrast
DIMP	Diisopropyl methylphosphonate
DL	Detection limit
DNA	Deoxyribonucleic acid
2,4-DNPH	2,4-Dinitrophenylhydrazine
DOC	U.S. Department of Commerce
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
DOT	U.S. Department of Transportation
DPD	N,N-Diethyl-p-phenylenediamine
DQO	Data quality objective
DTPA	Diethylenetriamine-pentaacetate
DVL	Detection verification level
EA2192	S-2-(Diisopropylamino)ethyl methylphosphonothioic acid
EC	*Escherichia coli*
ECD	Electron capture detector
e-CFR	Electronic Code of Federal Regulations
ECL	Electrochemiluminescence
ED	Ethyldichloroarsine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDEA	N-Ethyldiethanolamine
EDL	Estimated detection limit
EDTA	Ethylenediaminetetraacetic acid
EDXA	Energy dispersive X-ray analysis
ELFA	Enzyme-linked fluorescent immunoassay
ELISA	Enzyme-linked immunosorbent assay
EMC	Emission Measurement Center
EML	Environmental Measurements Laboratory
EMMI	Environmental Monitoring Methods Index
EMPA	Ethyl methylphosphonic acid
EMSL	Environmental Monitoring and Support Laboratory
EPA	U.S. Environmental Protection Agency
EQL	Estimated quantitation limit
ERLN	Environmental Response Laboratory Network
ESAM	Environmental Sampling and Analytical Methods (EPA)
ESI	Electrospray ionization
ESI-MS-MS	Electrospray ionization – tandem mass spectrometry
ETV	Environmental Technology Verification
FA	Immunofluorescence assay
FAA	Fluoroacetate anion
FBI	U.S. Federal Bureau of Investigation
FDA	U.S. Food and Drug Administration

FEMS	Federation of European Microbiological Societies
FGC-ECD	Fast gas chromatography with electron capture detection
FID	Flame ionization detector
FL	Fluorescence detector
FPD	Flame photometric detector
FRET	Forster resonance energy transfer
FRhK-4	Fetal rhesus monkey kidney
FRMAC	Federal Radiological Monitoring and Assessment Center
FSIS	Food Safety and Inspection Service
GA	Tabun
GB	Sarin
GC	Gas chromatograph or Gas chromatography
GC-ECD	Gas chromatography-electron capture detector
GC-FID	Gas chromatography-flame ionization detector
GC-FPD	Gas chromatography-flame photometric detector
GC-MS	Gas chromatography-mass spectrometry
GC-MS-TOF	Gas chromatography-mass spectrometry-time of flight
GC-NPD	Gas chromatography-nitrogen-phosphorus detector
GD	Soman
GE	1-Methylethyl ester ethylphosphonofluoridic acid
Ge	Germanium
Ge(Li)	Germanium (Lithium)
GF	Cyclohexyl sarin
GFAA	Graphite furnace atomic absorption spectrophotometer or Graphite furnace atomic absorption spectrophotometry
GTX	Gonyautoxins
HASL	Health and Safety Laboratory, currently known as National Urban Security Technology Laboratory (NUSTL)
HAV	Hepatitis A virus
HCoV	Human coronavirus
HD	Sulfur mustard / mustard gas; bis(2-chloroethyl) sulfide
HEPA	High-efficiency particulate air
HEV	Hepatitis E virus
HFBA	Heptafluorobutyric anhydride
HFBI	Heptafluorobutyrylimidazole
HHS	U.S. Health and Human Services
HILIC	Hydrophilic interaction liquid chromatography
HILIC-MS-MS	Hydrophilic interaction liquid chromatography-tandem mass spectrometry
HLB	Hydrophilic-lipophilic-balanced
HMTD	Hexamethylenetriperoxidediamine
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HN-1	Nitrogen mustard 1; bis(2-chloroethyl)ethylamine
HN-2	Nitrogen mustard 2; 2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine
HN-3	Nitrogen mustard 3; tris(2-chloroethyl)amine
HPGe	High purity germanium
HPLC	High performance liquid chromatography
HPLC-FL	High performance liquid chromatography-fluorescence
HPLC-MS	High performance liquid chromatography-mass spectrometry
HPLC-MS-MS	High performance liquid chromatography tandem mass spectrometry
HPLC-UV	High performance liquid chromatography-ultraviolet
HPLC-vis	High performance liquid chromatography-visible

HRP	Horseradish peroxidase
HSMMD	Homeland Security and Materials Management Division
HSRP	Homeland Security Research Program
HTO	Tritiated water
HV	High volume
IC	Ion chromatograph or Ion chromatography
ICLN	Integrated Consortium of Laboratory Networks
ICP	Intestinal contents preparation (pathogens); Inductively coupled plasma (chemistry)
ICP-AES	Inductively coupled plasma – atomic emission spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
I.D.	Inner diameter
IDL	Instrument detection limit
IMPA	Isopropyl methylphosphonic acid
IMS	Immunomagnetic separation
IO	Inorganic
IPR	Initial precision and recovery
IRIS	Integrated Risk Information System (EPA)
ISE	Ion specific electrode
ISG	Impregnated silica gel
ISM02.3	Inorganic Superfund Methods Multi-Media, Multi-Concentration ISM02.3
ISO	International Organization for Standardization
KHP	Potassium hydrogen phthalate
L-1	Lewisite 1; 2-Chlorovinyldichloroarsine
L-2	Lewisite 2; bis(2-Chlorovinyl)chloroarsine
L-3	Lewisite 3; tris(2-Chlorovinyl)arsine
LC	Liquid chromatograph or Liquid chromatography
LC-APCI-MS	Liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry
LC-ESI-MS	Liquid chromatography-electrospray ionization-mass spectrometry
LC-ESI-MS-MS	Liquid chromatography-electrospray ionization-tandem mass spectrometry
LCMRL	Lowest common minimum reporting level
LC-MS	Liquid chromatography-mass spectrometry
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LC-PIM-MS	Liquid chromatography-product ion monitoring-mass spectrometry
LC-UV	Liquid chromatography-ultraviolet
LD50	Median lethal dose
LFA	Lateral flow immunoassay
LFD	Lateral flow device
LLD	Lower limit of detection
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of quantitation
LRN	Laboratory Response Network
LSC	Liquid scintillation counter
LSE	Liquid-solid extraction
M	Molar
mAbs	Monoclonal antibodies
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser-desorption ionization
MALDI-TOF-MS	Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry
MARLAP	*Multi-Agency Radiological Laboratory Analytical Protocols*
MC	Microcystin
MDC	Minimum detectable concentration

MDCK	Madin-Darby canine kidney cells
MDEA	N-Methyldiethanolamine
MDL	Method detection limit
MFA	Methyl fluoroacetate
MIC	Methyl isocyanate
mLD_{50}	Mouse lethal dose
MPA	Methylphosphonic acid
MRL	Minimum reporting level
MRM	Multiple reaction monitoring
mRNA	Messenger ribonucleic acid
MS	Mass spectrometer or Mass spectrometry
MS-MS	Tandem mass spectrometry
MS/MSD	Matrix spike/Matrix spike duplicate
MSE	Microscale solvent extraction
MTBE	Methyl *tert*-butyl ether
MW	Molecular weight
MWCO	Molecular weight cut-off
NA	Not applicable
NaI(Tl)	Thallium-activated sodium iodide
NAREL	National Air and Radiation Environmental Laboratory
NBD chloride	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole
NCPDCID	National Center for the Prevention, Detection, and Control of Infectious Diseases
NCRP	National Council on Radiation Protection and Measurements
NEMI	National Environmental Methods Index
NEO	Neosaxitoxins
NERL	National Exposure Research Laboratory (EPA)
NHSRC	National Homeland Security Research Center (EPA)
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
nM	Nanomolar
NMAM	NIOSH Manual of Analytical Methods
NNSA	National Nuclear Security Administration
NOD	Nodularins
NPD	Nitrogen-phosphorus detector
NRC	U.S. Nuclear Regulatory Commission
NRMRL	National Risk Management Research Laboratory (EPA)
nS	Nano siemens
NTIS	National Technical Information Service
NTU	Nephelometric turbidity units
OAQPS	Office of Air Quality Planning and Standards (EPA)
OAR	Office of Air and Radiation (EPA)
OGWDW	Office of Water, Office of Ground Water and Drinking Water (EPA)
OLEM	Office of Land and Emergency Management (EPA)
OPR	Ongoing precision and recovery
ORAU	Oak Ridge Associated Universities
ORD	Office of Research and Development (EPA)
ORIA	Office of Radiation and Indoor Air (EPA)
ORISE	Oak Ridge Institute for Science and Education
OSHA	Occupational Safety and Health Administration
OVS	OSHA versatile sampler
OW	Office of Water (EPA)
PCDDs	Polychlorinated dibenzo-p-dioxins

PCDFs	Polychlorinated dibenzofurans
PCR	Polymerase chain reaction
PEL	Permissible exposure limit
PETN	Pentaerythritol tetranitrate
PFBHA	O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine
PFE	Pressurized fluid extraction
PHILIS	Portable High Throughput Integrated Laboratory Identification Systems
PIM	Product ion monitoring
PLOS	Public Library of Science
PLRP-S	Polymeric reversed phase
PMPA	Pinacolyl methyl phosphonic acid
1,2-PP	1-(2-Pyridyl)piperazine
PP2A	Protein Phosphatase 2A
ppbv	Parts per billion by volume
pptv	Parts per trillion by volume
PST	Paralytic shellfish toxin
PTFE	Polytetrafluoroethylene
PUF	Polyurethane foam
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
QA	Quality assurance
QAP	Quality assessment program
QAPP	Quality assurance project plan
QC	Quality control
QL	Quantitation limit
qPCR	Quantitative polymerase chain reaction
R 33	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (VR)
RBA	Receptor binding assay
RCRA	Resource Conservation and Recovery Act
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RESL	Radiological and Environmental Sciences Laboratory
RFV	Relative fluorescence value
RLAB	Regional laboratory method
RLU	Relative light units
RNA	Ribonucleic acid
RNAse	Ribonuclease
rRNA	Ribosomal ribonucleic acid
RSD	Relative standard deviation
RTECS	Registry of Toxic Effects of Chemical Substances
RTG	Radioisotope thermoelectric generator
RT-PCR	Reverse transcription-polymerase chain reaction
RT-qPCR	Quantitative reverse transcription-polymerase chain reaction
RV-PCR	Rapid viability-polymerase chain reaction
RV-RT-PCR	Rapid viability-reverse transcription-polymerase chain reaction
SAED	Select area electron diffraction
SAM	*Selected Analytical Methods for Environmental Remediation and Recovery*
SAP	Sampling and analysis plan
SARS	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2 (COVID-19)
SCID	Sample Collection Information Document
SEA	Staphylococcal enterotoxin type A
SEB	Staphylococcal enterotoxin type B

SEC	Staphylococcal enterotoxin type C
SED	Staphylococcal enterotoxin type D
SEE	Staphylococcal enterotoxin type E
SET	Staphylococcal enterotoxin
SIM	Selective ion monitoring
SIS	Selected ion storage
SM	*Standard Methods for the Examination of Water and Wastewater*
SOP	Standard operating procedure
SOW	Statement of work
SPE	Solid-phase extraction
SPR	Solid-phase receptacle
SRC	Syracuse Research Corporation
SRM	Single reaction monitoring
SRS	Savannah River National Laboratory, Savannah River Site
STEC	Shiga-toxigenic *E. coli*
STEL	Short term exposure limit
STS	Sample test source
STX	Saxitoxin
Stx	Shiga toxin
Stx-1	Shiga toxin Type 1
Stx-2	Shiga toxin Type 2
SW	Solid waste
T_0	Time zero
T_2O	Tritium oxide
TBD	To be determined
TCLP	Toxicity Characteristic Leaching Procedure
TDG	Thiodiglycol
TEA	Triethanolamine
TEM	Transmission electron microscope or Transmission electron microscopy
TEPP	Tetraethyl pyrophosphate
TETS	Tetramethylenedisulfotetramine or tetramine
T_f	Time final
THF	Tetrahydrofuran
TIC	Total ion chromatogram
TIOA	Tri-isooctylamine
1,3,5-TNB	1,3,5-Trinitrobenzene
2,4,6-TNT	2,4,6-Trinitrotoluene
TO	Toxic Organic
TOF	Time-of-flight
TOF-MS	Time-of-flight mass spectrometry
TOPO	Trioctylphosphine oxide
TOXNET	Toxicology Data Network
TRF	Time-resolved fluorescence
TRU	Transuranic
TTX	Tetrodotoxin
UF	Ultrafiltration
UPLC	Ultra performance liquid chromatography
U.S.	United States
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
UV	Ultraviolet
VBNC	Viable but non-culturable

VC	*Vibrio cholerae*
VCSB	Voluntary Consensus Standard Body
VE	Phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester
VG	Phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester
vis	Visible detector
VM	Phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester
VOA	Volatile organic analysis
VOC	Volatile organic compound
VR	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (R 33)
VX	O-Ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate
WCIT	Water Contaminant Information Tool
WEF	Water Environment Federation
WHO	World Health Organization
WLA	Water Laboratory Alliance
WSD	Water Security Division (EPA, Office of Water)
YP	*Yersinia pestis*

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Executive Summary

The U.S. Environmental Protection Agency's (EPA's) Selected Analytical Methods for Environmental Remediation and Recovery (SAM) represents the latest step in an ongoing effort of EPA's Homeland Security Research Program (HSRP) to provide selected analytical methods to laboratories tasked with analyzing environmental samples in support of EPA remediation and recovery efforts following an intentional or accidental homeland security-related contamination incident. The information is intended for use by EPA and EPA-contracted and -subcontracted laboratories; it also can be used by other agencies and laboratory networks and as a tool to assist state and local laboratories in planning for and analyzing chemical, biological and/or radiological (CBR) environmental samples and radioactively contaminated outdoor building material samples. The information also can be found on the Environmental Sampling and Analytical Methods (ESAM) Program website via the *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM) webpage, which provides a searchable query tool for users to access supporting information regarding selected methods.

Although not all of the selected methods have been validated at this time, they are considered to contain the most appropriate currently available techniques, based on expert judgment of the SAM technical work groups. Usability tiers have been assigned to the methods selected for chemical, pathogen and biotoxin analytes to provide an indication of method applicability (i.e., the extent to which the methods have been tested and applied for analysis of the specific analyte and sample type for which they have been selected). Method usability tiers are not assigned to methods that address radiochemistry analytes. Unless a method states applicability to a specific analyte/sample type, it should be assumed that method evaluation is needed, and adjustments may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences and data quality objectives (DQOs).

EPA strives to continue development and evaluation of analytical methods and protocols, including optimization of procedures for measuring target analytes or agents in specific sample types, as appropriate. In cases where method procedures are determined to be insufficient for a particular situation, HSRP will continue to provide technical support regarding appropriate actions. HSRP has also compiled information and published documents regarding sample collection, rapid screening/preliminary identification equipment, and disposal of samples corresponding to the analytes and sample types addressed in this document. This information is available on the SAM Companion Sample Collection Information Documents (SCIDs) webpage and Sample Collection Procedures and Strategies webpage.

Product Development Quality Assurance

The information in this document is based on secondary sources, including peer-reviewed scientific methods, manuals and publications; federal agency websites; industry providers of equipment and materials (i.e., vendors); and nationally-recognized scientific, technical or response organizations. Full citations and links to each method and cited publication are provided throughout the document.

The document completed several review cycles prior to publication, including EPA project lead review, technical work group reviews, internal EPA technical review, Homeland Security and Materials Management Division (HSMMD) quality assurance and technical edit reviews, external technical review, and HSMMD management reviews. All comments from reviewers have been tracked and are maintained by EPA, along with the revisions and adjustments made to address the comments.

Section 1.0: Introduction

After the terrorist attacks of September 11, 2001 and the anthrax attacks in the fall of 2001, federal and state personnel provided response, recovery and remediation under trying circumstances, including unprecedented demand on laboratory capabilities to analyze environmental samples. Caused naturally or by humans, environmental emergencies continue to challenge our Nation. The use of chemical threats world-wide and several recent water system contamination incidents, such as the 2014 industrial storage tank leak into West Virginia's Elk River, remind us of the impact that contaminants can have on public health. Radiological contamination following the Fukushima Daiichi nuclear disaster in 2011 demonstrated the significant impact and challenge of cleaning up large-scale contamination. Smaller-scale incidents such as attempted ricin poisonings in several communities around the country highlight the ever-present threat of terrorism post 2001. Natural disasters such as the 2014 microcystins contamination of drinking water in Toledo, Ohio, continue to threaten and damage water systems and infrastructure, leading to contamination and waterborne disease. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic and opioid crisis (e.g., fentanyl) have resulted in public health concerns due to environmental contamination of air and surfaces.

Following the 2001 attacks, the U.S. Environmental Protection Agency (EPA) identified several areas to enhance the resiliency of the Nation following homeland security-related incidents resulting in contamination.[1] The need to improve the Nation's laboratory capacity and capability to analyze environmental samples following such incidents was one of the most important areas identified and remains so today. To address these needs, EPA formed the Homeland Security Laboratory Capacity Work Group, charged with identifying and implementing opportunities for near-term improvements and to develop recommendations for addressing longer-term laboratory issues. A critical area identified was the need for a list of selected analytical methods to be used by all laboratories when analyzing contamination incident samples and, in particular, when analysis of a large number of samples is required over a short period of time.

Since 2004, EPA, through its Homeland Security Research Program (HSRP), has brought together workgroups consisting of technical experts from across EPA and other interested agencies to address site characterization, remediation and clearance following homeland security-related contamination incidents, and to develop this compendium of analytical methods to be used when analyzing environmental samples, which is now referred to as EPA's *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM) [2,3]. Participants in the SAM technical workgroups have included representatives from EPA program offices, regions, and laboratories, including the Offices of Research and Development (ORD), Air and Radiation (OAR), Water (OW), Land and Emergency Management (OLEM), Environmental Information, and Chemical Safety and Pollution Prevention. Technical workgroups have also included participants from the U.S. Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), Department of Homeland Security (DHS), Federal Bureau of Investigation (FBI), Department of Defense (DoD), Department of Energy (DOE), Department of Agriculture (USDA), Geological Survey (USGS) and Department of Commerce (DOC), as well as other federal, state and local agencies, public utilities, municipalities and universities. Many work group members work closely with

[1] For the purposes of SAM, homeland security-related incidents encompass man-made contamination (whether intentional or unintentional), natural disasters and epidemics that impact or threaten the safety, security and resiliency of the United States.

[2] This document was developed in accordance with the quality objectives outlined in the project's quality assurance project plan.

[3] Formerly EPA's *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events*. SAM and its methods are available at: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

EPA's Environmental Response Laboratory Network (ERLN)[4], a national network of laboratories that can be accessed as needed to support responses to large-scale environmental contamination incidents, and the Water Laboratory Alliance (WLA)[5], which can be accessed specifically for responses pertaining to the Nation's water sector.

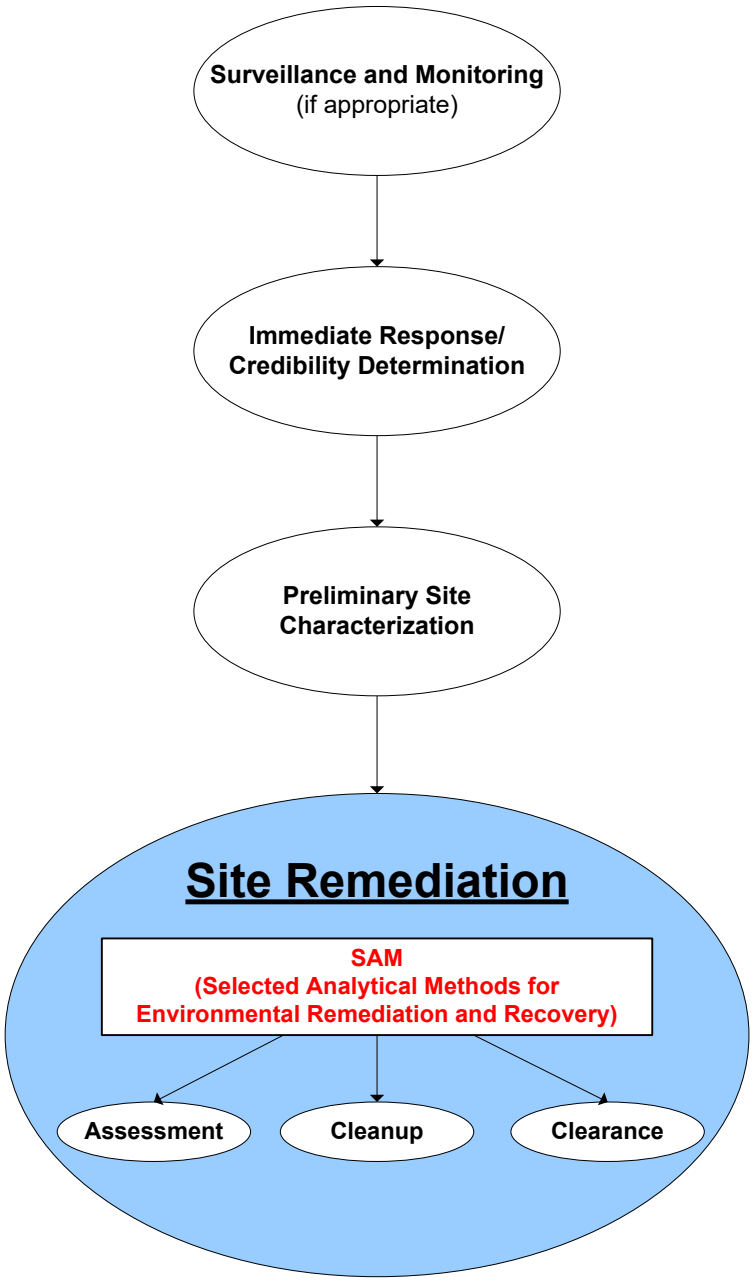
Widely different analytical methods might be required for various phases of environmental sample analyses in support of homeland security preparedness and response—for example, during: (1) ongoing surveillance and monitoring; (2) response and credibility determination, to determine whether an incident has occurred; (3) preliminary site characterizations to determine the extent and type of contamination; and (4) confirmatory laboratory analyses to support site assessment, cleanup and clearance decisions during site remediation. **Figure 1-1** represents these analytical phases.

SAM provides information for analytical methods to be applied during the "Site Remediation" phase. Methods have been selected to support activities related to site assessment (including preliminary, qualitative analyses to characterize the extent of contamination), site cleanup (to evaluate the efficacy of remediation efforts), and site clearance (releasing the remediated area for its intended use) decisions.

[4] Information regarding EPA's Environmental Response Laboratory Network (ERLN) is available at: https://www.epa.gov/emergency-response/environmental-response-laboratory-network

[5] Information regarding the Water Laboratory Alliance (WLA) is available at: https://www.epa.gov/waterlabnetwork

Figure 1-1. Environmental Evaluation Analytical Process Roadmap for Homeland Security Incidents



Note: Sites undergoing remediation will vary in size, location and type, and are defined in site- and incident-specific documentation (e.g., sample collection plan, sampling and analysis plan, quality assurance project plan).

Methods and protocols are considered for chemical, radiochemical, biological and biotoxin agents of concern in the types of environmental samples that would be anticipated, including outdoor building and infrastructure materials containing radiochemical contamination. Work groups also have been considering methods that might be needed to address waste generated during site decontamination and the analytical impacts of decontamination agents.

Surveys of available analytical methods are conducted using existing resources, including the following:

- National Environmental Methods Index (NEMI) and NEMI for Chemical, Biological and Radiological Methods (NEMI-CBR)
- Environmental Monitoring Method Index (EMMI)
- EPA Test Methods Index
- EPA Office of Water Methods
- EPA Office of Solid Waste SW-846 Methods
- EPA HSRP/CESER Methods
- EPA Office of Radiation and Indoor Air (ORIA) Methods
- EPA Standard Operating Procedures (SOPs)
- FDA Methods
- USDA Methods
- National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods (NMAM)
- Occupational Safety and Health Administration (OSHA) Index of Sampling and Analytical Methods
- AOAC International
- ASTM International
- International Organization for Standardization (ISO) methods
- *Standard Methods for the Examination of Water and Wastewater* (SM)
- Scientific Literature

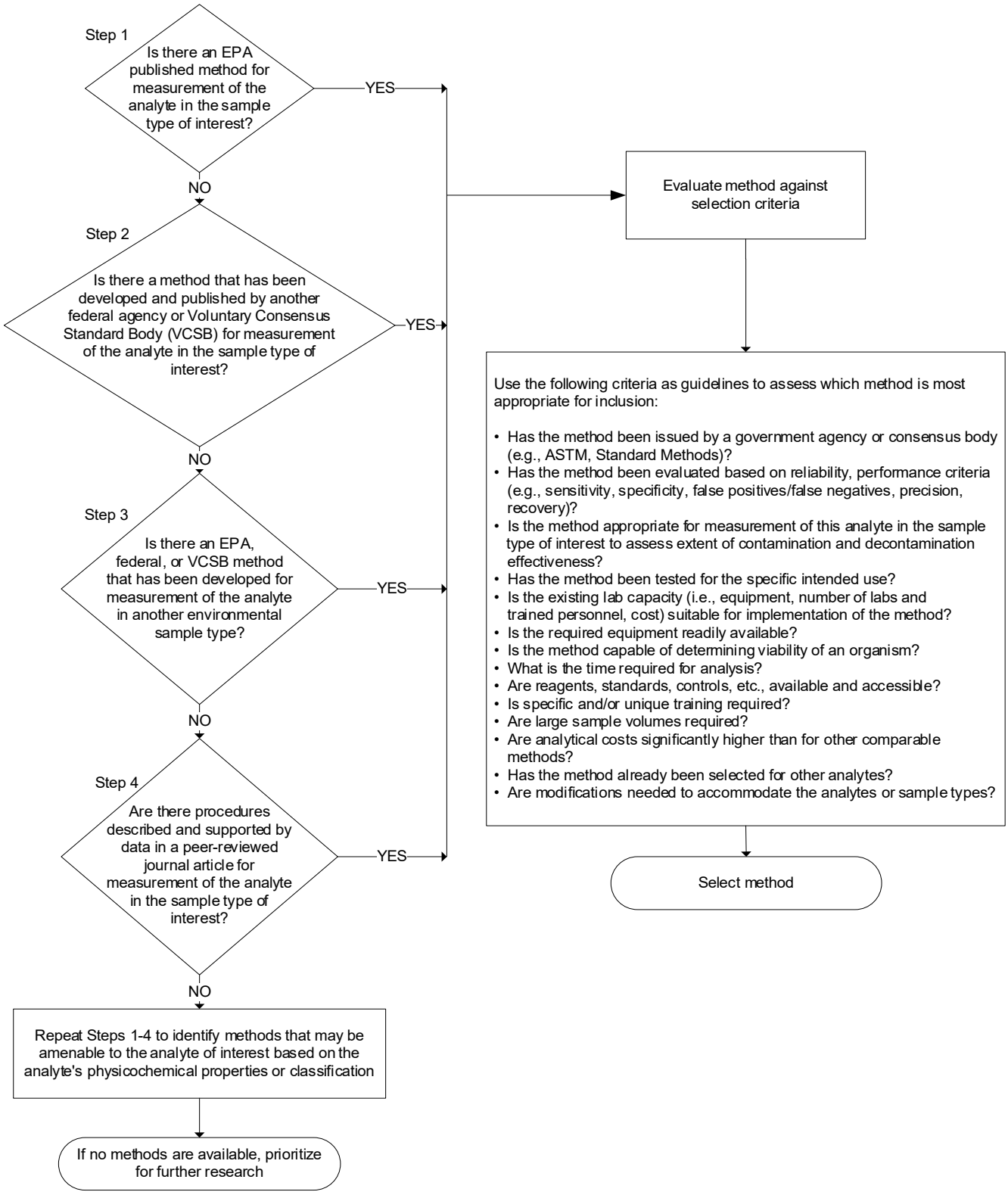
Section 2.0: Background

SAM technical work groups are charged with selecting methods as appropriate, determining method tier classifications, providing input into special considerations, and adding or removing analytes of interest. Work groups identify a single method or method group for each analyte/sample type. The goals of selecting these methods for use by multiple laboratories during an incident include increasing analytical efficiency, permitting sharing of sample loads between laboratories, improving data comparability, and simplifying the task of outsourcing analytical support to the commercial laboratory sector. Use of such methods also can improve follow-up activities, including validating results, evaluating data and making decisions. Details regarding changes that have been incorporated into each revision of SAM are provided in Attachment 1.

SAM analytes are selected based on criteria (e.g., environmental persistence, half-lives, availability and toxicity) that address the needs and priorities of EPA as well as other federal agencies. The sample types addressed are specific to each technical section and have been determined by the technical work groups to be a concern during site remediation. SAM work groups select methods based on consideration of criteria that emphasize method performance and include existing laboratory capabilities, laboratory capacity, method applicability to multiple sample types, and method applicability to multiple analytes. For some analytes, the preferred method is a clear choice; for others, competing criteria make the choice difficult. Final method selections are based on technical recommendations from the work groups under the direction of EPA's HSRP. For analytes where methods or laboratory capabilities are limited, methods are selected that may be amenable to the analyte of interest based on the analyte's physicochemical properties or classification. In these cases, laboratory studies to evaluate the ability of the method to measure the target analyte(s) are either underway or needed.

Figure 2-1 summarizes steps and provides the criteria used during the method selection process. It is important to note that the method selection criteria are listed in non-hierarchical order and, in some cases, only a subset of the criteria was considered when selecting methods.

Figure 2-1. Method Selection Process



Note: Voluntary Consensus Standards Bodies (VCSBs) include organizations such as ASTM International, ISO, AOAC International, and Standard Methods.

The primary objective of SAM is to support EPA's ERLN and WLA by identifying methods that provide documented analytical techniques and produce consistent results of known quality. Although ideally methods would provide documented analytical techniques and produce consistent results of known quality, it is not possible for the selected methods to do both in all cases. For some analyte/sample type pairs, for example, SAM work group members have been able to identify journal articles that do not include specific detailed techniques. In other cases, the analytical methods selected do not include quality control specifications or criteria.

Although not all the selected methods have been validated at this time, they are considered to contain the most appropriate currently available techniques, based on expert judgment of the SAM technical work groups. Method usability tiers (i.e., the extent to which the methods have been tested and applied for analysis of the specific analyte and sample type(s) for which they have been selected) are assigned to methods that have been selected to address the chemical, pathogen and biotoxin analytes. Method usability tiers are not assigned to methods that address radiochemistry analytes. Unless a published method states specific applicability to the analyte/sample type for which it has been selected, it should be assumed that method evaluation is needed, and adjustments to the procedures may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences, variations in the purity and availability of reference standards, and data quality objectives (DQOs). Where further development and testing are necessary, EPA is continuing to develop and evaluate analytical techniques based on the methods and protocols that are listed in this document and based on current EPA policies for validating analytical methods. Once validation is complete, data regarding method performance and DQOs will be made available.

EPA recognizes that selection of a single method might limit laboratory capability and affect laboratory capacity when techniques that differ from those provided in the methods are required for analysis of difficult samples. In those cases, EPA will continue to provide technical support regarding appropriate actions (see list of contacts in Section 4.0). Additional information is provided in the Agency Policy Directive Number FEM-2010-01.[6] EPA also recognizes that selection of methods prior to the occurrence of specific contamination incidents may result in some limitations, including the following:

- Selecting technologies that may not be the most cost-effective for addressing a particular situation;
- Selecting methodologies that may not be appropriate for use in responding to a particular incident because EPA did not anticipate having to analyze for a particular analyte or analyte/sample type combination; and
- Discouraging use of new and better measurement technologies.

With these limitations in mind, and towards the goal of preparedness, SAM work groups have evaluated the suitability of existing methodologies and selected this set of methods for use by laboratories that will be called on to support EPA environmental remediation efforts following an intentional or unintentional contamination incident. Work groups took the following measures during method selection:

- Using an established method selection process (Figure 2-1) to help ensure that the analytical methods listed provide results that are consistent with and support their intended use;
- Including members of the Integrated Consortium of Laboratory Networks (ICLN), which includes the ERLN and WLA, in SAM work groups to ensure that the selected methods meet the network's

[6] U.S. EPA, Forum on Environmental Measurements, July 21, 2010, *Ensuring the Validity of Agency Methods Validation and Peer Review Guidelines: Methods of Analysis Developed for Emergency Response Situations,* Agency Policy Directive Number FEM-2010-01. https://www.epa.gov/sites/production/files/2015-01/documents/emergency_response_validity_policy.pdf

needs for consistent analytical capabilities, to address capacity, and to provide quality data to inform remediation decisions; and

- Continuing to work with multiple agencies and stakeholders to update methods and protocols, as needed.

Section 3.0: Scope and Application

SAM represents the latest step in an ongoing effort by EPA's HSRP to provide selected analytical methods for use in cases when multiple laboratories are called on to analyze environmental samples and radioactively contaminated outdoor building material samples in support of EPA remediation and recovery efforts following an intentional or accidental homeland security-related contamination incident. The information is intended for use by EPA and EPA-contracted and -subcontracted laboratories, such as laboratory members of the ERLN and WLA. It can also be used by other agencies and laboratory networks and as a tool to assist state and local laboratories. The methods should be used to support the following during site remediation:

- Assessment: Determine the extent of site contamination (assumes early responders have identified contaminants prior to EPA's remediation effort)
- Cleanup: Assess the remediation efforts during the site cleanup process
- Clearance: Confirm the effectiveness of decontamination in support of site clearance decisions

The selected methods correspond to specific analyte/sample type combinations that are listed in Appendices A (chemical), B (radiochemical), C (pathogen) and D (biotoxin). Summaries of each method are provided throughout Sections 5.2 (chemical methods), 6.2 and 6.3 (radiochemical methods), 7.2 (pathogen methods) and 8.2 (biotoxin methods). The information also can be found on the SAM webpage, which provides a searchable query tool for users to access supporting information regarding the selected methods. The methods are limited to those that would be used to determine, to the extent possible within analytical limitations, the presence of chemical, radiochemical, pathogen and biotoxin analytes of concern and their concentrations and activity/viability, when applicable, in environmental media and radiochemical analytes of concern in outdoor building materials. The majority of methods include detailed laboratory procedures for confirming the identification of analytes and determining their concentrations in samples and, therefore, are not designed to be used for rapid or immediate response or for conducting an initial evaluation.

EPA plans to continue to update SAM as appropriate to address the needs of homeland security, to reflect improvements in analytical methodology and new technologies, and to incorporate changes in analytes based on needs. The methods that have been selected for each analyte/sample type combination were deemed the most general, appropriate, and broadly applicable of available methods by work groups consisting of technical experts in each field, and are subject to change following further research to improve methods or following the development of new methods. EPA also periodically provides addenda to provide updates regarding methods, information and issues that are not addressed by the most current versions of SAM, and the contacts listed in Section 4.0 encourage the scientific community to inform them of any such method improvements.

SAM is not intended to provide information regarding sample collection activities or equipment. In addition to updating selected analytes and methods, SAM work group members have developed companion documents to provide information regarding sample collection, rapid screening and preliminary analysis equipment, and sample disposal to supplement the selected analytical methods. The information in the companion documents generally corresponds to the SAM analytes and methods and the documents are updated as needed and as resources allow. Currently available HSRP-developed companion documents are listed below and, with content descriptions, in Attachment 1.

- *Field Application of Emerging Composite Sampling Methods*
- *Guide for Development of Sample Collection Plans for Radiochemical Analytes in Environmental Matrices Following Homeland Security Events*

- *Guide for Development of Sample Collection Plans for Radiochemical Analytes in Outdoor Infrastructure and Building Materials Following Homeland Security Incidents*
- *Laboratory Analytical Waste Management and Disposal Document – Companion to Selected Analytical Methods for Environmental Remediation and Recovery*
- *Rapid Screening and Preliminary Identification Techniques and Methods – Companion to SAM Revision 5.0*
- *Sample Collection Information Documents (SCIDs)*
- *Sample Collection Procedures for Radiochemistry Analytes in Environmental Matrices*
- *Sample Collection Procedures for Radiochemistry Analytes in Outdoor Building and Infrastructure Materials*
- *Sample Collection Protocol for Bacterial Pathogens in Surface Soil*
- *Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field*
- *Collection of Microbiological Agent Samples from Potentially Contaminated Porous Surfaces Using Microvacuum Techniques*
- *Collection of Surface Samples Potentially Contaminated with Microbiological Agents Using Swabs, Sponge Sticks and Wipes*
- *Collection of Air Samples Potentially Contaminated with Microbiological Agents Using Impingers, Impactors and Low-Volume Filters*
- *Sampling and Analysis Plan (SAP) Template Tool for Addressing Environmental Contamination by Pathogens* and corresponding *User Guide*

EPA recognizes that having data of known and documented quality is critical in making proper decisions and strives to establish site-specific DQOs for each response activity.[7] These DQOs are based upon needs for both quality and response time. Many of the methods listed in SAM include QC requirements for collecting and analyzing samples. These QC requirements may be adjusted as necessary to maximize data and decision quality. Specific QC considerations and recommendations for analysis of samples for chemical, radiochemical, pathogen and biotoxin analytes are provided in each corresponding section of this document (i.e., Sections 5.1.2, 6.1.2, 7.1.2 and 8.1.2, respectively). EPA's ERLN, which is tasked with providing laboratory support following intentional or unintentional environmental contamination incidents, also has established data reporting procedures. Requirements for receiving, tracking, storing, preparing, analyzing and reporting data are specified in the U.S. EPA (2011) *Environmental Response Laboratory Network Laboratory Requirements Document*; project-specific requirements also are included in individual Analytical Service Requests (ASRs).

[7] Information regarding EPA's DQO process, considerations, and planning is provided in EPA's *Guidance on Systematic Planning Using the Data Quality Objectives Process*, EPA QA/G-4.

Section 4.0: Points of Contact

Questions concerning this document, or the methods identified in this document, should be addressed to the appropriate point(s) of contact identified below. EPA recommends that these contacts be consulted regarding any method deviations or modifications, sample problems or interferences, QC requirements, the use of potential alternative methods, or the need to address analytes or sample types other than those listed. As previously indicated, any deviations from the recommended method(s) should be reported immediately to ensure data comparability is maintained when responding to intentional or unintentional contamination incidents. In cases where laboratories are specifically tasked by EPA to use these methods following an incident, method deviations or modifications must be approved by the Analytical Service Requestor (as defined by ERLN) prior to use. In addition, general questions and comments can be submitted via the SAM webpage.

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Section 5.0: Selected Chemical Methods

Appendix A provides a list of methods to be used in analyzing environmental samples for chemical contaminants during remediation activities that result from a contamination incident. Methods are listed for each analyte and for each sample type that may need to be measured and analyzed when responding to an environmental contamination incident. In some cases, procedures from peer-reviewed journal articles or provisional methods are listed for those analyte-sample type combinations where validated methods are unavailable. In these instances, the best available procedure was selected based on its environmental application and on data quality objectives (DQOs). Appendix A includes method usability tiers that have been assigned to each method to indicate its applicability to the specific analyte-sample type combination(s) for which it has been selected. These tiers are described in Section 5.1.1 below, and are defined on the first page of Appendix A. As appropriate, when fully validated methods become available, the literature references and alternative methods will be replaced.

Please note: This section provides guidance for selecting chemical methods to facilitate data comparability when laboratories are faced with a large-scale environmental restoration crisis. Not all methods have been verified for the analyte/sample type combinations listed in Appendix A. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix A is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The component, contaminant or constituent of interest.
- **Chemical Abstracts Service Registry Number (CAS RN** [Chemical Abstracts Service, Columbus, OH]**).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names.
- **Determinative technique.** An analytical instrument or technique used to determine the quantity and identification of compounds or components in a sample.
- **Method type.** Two method types (sample preparation and determinative) are used to complete sample analysis. In some cases, a single method contains information for both sample preparation and determinative procedures. In most instances, however, two separate methods may need to be used in conjunction.
- **Solid samples.** The recommended method / procedure to identify and measure the analyte of interest in solid-phase samples.
- **Non-drinking water samples.** The recommended method / procedure to identify and measure the analyte of interest in aqueous liquid-phase samples other than drinking water.
- **Drinking water samples.** The recommended method / procedure to identify and measure the analyte of interest in drinking water samples.
- **Air samples.** The recommended method / procedure to identify and measure the analyte of interest in air samples.
- **Wipe samples.** The recommended method / procedure to identify and measure the analyte of interest in wipes used to collect a sample from a surface.

Following an environmental contamination incident, it is assumed that only those areas with contamination greater than pre-existing / naturally prevalent levels commonly found in the environment would be subject to remediation. Dependent on site- and incident-specific goals, investigation of background levels using methods listed in Appendix A is recommended.

5.1 General Guidelines

This section provides a general overview of how to identify the appropriate chemical method(s) for a given analyte-sample type combination, as well as recommendations for quality control (QC) procedures.

The following resources are available for additional information on the properties of the chemicals listed in Appendix A:

- Syracuse Research Corporation's (SRC) PHYSPROP (http://www.srcinc.com/what-we-do/environmental/scientific-databases.html) contains information pertaining to chemical structures, names, physical properties and persistence. PHYSPROP is sponsored by the U.S. Environmental Protection Agency (EPA), and is included in EPA's Estimation Program Interface (EPI) Suite™.
- INCHEM (http://www.inchem.org/) contains both chemical and toxicity information.
- The Registry of Toxic Effects of Chemical Substances (RTECS) database can be accessed via the National Institute for Occupational Safety and Health (NIOSH) website (http://www.cdc.gov/niosh/rtecs/default.html) for toxicity information.
- EPA's Integrated Risk Information System (IRIS) (http://www.epa.gov/iris/) contains toxicity information.
- EPA's Water Contaminant Information Tool (WCIT) (https://www.epa.gov/waterdata/water-contaminant-information-tool-wcit) can be accessed by registered users.
- *Forensic Science and Communications* (http://www.fbi.gov/about-us/lab/forensic-science-communications) is published by the Laboratory Division of the Federal Bureau of Investigation (FBI).
- Joint Research Centre / Institute for Health & Consumer Protection (https://ec.europa.eu/jrc/en) contains information regarding European Directive 67/548/EEC and Annex V.
- Agency of Toxic Substances & Disease Registry (ATSDR) Toxic Substances Portal (http://www.atsdr.cdc.gov/toxprofiles/index.asp) provides Toxicological Profiles.
- Chemical Safety Data Sheets (http://www.ilpi.com/msds/).
- The National Institutes of Health's PubChem (https://pubchem.ncbi.nlm.nih.gov/) is an open chemistry database with information on chemicals such as chemical structures, toxicity data and chemical and physical properties.

In some cases, the availability of standards required for the selected analytical methods might be limited. In these cases, the chemistry methods points of contact listed in Section 4.0 should be contacted for additional information.

Some of the metal-containing analytes listed in SAM have been assigned selected methods that detect and measure only the metal component at this time. The goal is to eventually develop or identify appropriate methods that can be used to determine and measure the specific compounds. In the meantime, SAM assumes a contaminant is known once SAM analytical methods are applied, and identification and measurement of the metal provides an indication of the amount of contaminant present.

5.1.1 Standard Operating Procedures for Identifying Chemical Methods

The fitness of a method for an intended use is related to site-specific DQOs for a particular environmental remediation activity. These selected chemical methods have been assigned tiers (below) to indicate a level

of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate QC:

Tier I: Analyte/sample type is a target of the method(s). Data are available for all aspects of method performance and QC measures supporting its use for analysis of environmental samples following a contamination event. Evaluation and/or use of the method(s) in multiple laboratories indicate that the method can be implemented with no additional modifications for the analyte/sample type.

Tier II: (1) The analyte/sample type is a target of the method(s) and the method(s) has been evaluated for the analyte/sample type by one or more laboratories, or (2) the analyte/sample type is not a target of the method(s), but the method(s) has been used by laboratories to address the analyte/sample type. In either case, available data and/or information indicate that modifications will likely be needed for use of the method(s) to address the analyte/sample type (e.g., due to potential interferences, alternate matrices, the need to address different DQOs).

Tier III: The analyte/sample type is not a target of the method(s), and/or no reliable data supporting the method's fitness for its intended use are available. Data from other analytes or sample types, however, suggest that the method(s), with significant modification, may be applicable.

To determine the appropriate method to be used on an environmental sample, locate the analyte of concern under the "Analyte(s)" column in Appendix A: Selected Chemical Methods. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., high performance liquid chromatography [HPLC], gas chromatography-mass spectrometry [GC-MS]), then identify the appropriate sample preparation and determinative method(s) for the sample type of interest (solid, water, air or wipe). In some cases, two methods (sample preparation and determinative) are needed to complete sample analysis.

Once a method has been identified in Appendix A, **Table 5-1** can be used to locate the method summary. Sections 5.2.1 through 5.2.120 below provide summaries of the sample preparation and determinative methods listed in Appendix A.

Table 5-1. Chemical Methods and Corresponding Section Numbers

Analyte	CAS RN	Method	Section
A-230 Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate	2387496-12-8	L-A-507 Rev. 3 (EPA SOP)	5.2.62
A-232 Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate	2387496-04-8	L-P-107 Rev. 3 (EPA SOP)	5.2.63
A-234 Ethyl N-[(1E)-1-(diethylamino)ethylidene]-phosphoramidofluoridate	2387496-06-0	TO-17 (EPA ORD)	5.2.49
Acephate	30560-19-1	538 (EPA OW)	5.2.11
		J. Env. Sci. Health (2014) 49: 23–34	5.2.113
		J. Chromatogr. A (2007) 1154(1): 3–25	5.2.114

Analyte	CAS RN	Method	Section
Acrylamide	79-06-1	3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8316 (EPA SW-846)	5.2.38
		PV2004 (OSHA)	5.2.89
Acrylonitrile	107-13-1	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.24
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		8290A Appendix A (EPA SW-846)	5.2.36
		PV2004 (OSHA)	5.2.89
Aldicarb (Temik) Aldicarb sulfone Aldicarb sulfoxide	116-06-3 1646-88-4 1646-87-3	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.70
		D7645-16 (ASTM)	5.2.97
Allyl alcohol	107-18-6	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
4-Aminopyridine	504-24-5	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Ammonia	7664-41-7	350.1 (EPA OW)	5.2.6
		6016 (NIOSH)	5.2.75
		4500-NH_3 B (SM)	5.2.103
		4500-NH_3 G (SM)	5.2.104
Ammonium metavanadate (analyze as total vanadium) Arsenic, Total Arsenic trioxide (analyze as total arsenic)	7803-55-6 7440-38-2 1327-53-3	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80

Analyte	CAS RN	Method	Section
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		6001 (NIOSH)	5.2.71
		9102 (NIOSH)	5.2.80
Asbestos	1332-21-4	D5755-09(e1) (ASTM)	5.2.91
		D6480-19 (ASTM)	5.2.92
		10312:1995 (ISO)	5.2.101
Boron trifluoride	7637-07-2	ID216SG (OSHA)	5.2.88
Brodifacoum Bromadiolone	56073-10-0 28772-56-7	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		D7644-16 (ASTM)	5.2.96
BZ [Quinuclidinyl benzilate]	6581-06-2	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
		J. Chromatogr. B (2008) 874: 42–50	5.2.117
Calcium arsenate (analyze as total arsenic)	7778-44-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Carbofuran (Furadan)	1563-66-2	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.70
		D7645-16 (ASTM)	5.2.97
Carbon disulfide	75-15-0	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48

Analyte	CAS RN	Method	Section
Carfentanil	59708-52-0	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		L-A-309 Rev. 0 (EPA SOP)	5.2.60
		L-A-310 Rev. 1 (EPA SOP)	5.2.61
		J. Chromatogr. B (2014) 962: 52–58	5.2.119
Chlorfenvinphos	470-90-6	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Chlorine	7782-50-5	4500-Cl G (SM)	5.2.105
		Analyst (1999) 124(12): 1853–1857	5.2.106
2-Chloroethanol	107-07-3	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		2513 (NIOSH)	5.2.66
3-Chloro-1,2-propanediol	96-24-2	TO-10A (EPA ORD)	5.2.47
		Eur. J. Lipid Sci. Technol. (2011) 113: 345–355	5.2.109
		J. Chromatogr. A (2000) 866: 65–77	5.2.111
Chloropicrin	76-06-2	551.1 (EPA OW)	5.2.14
		EPA/600/R-16/114	5.2.55
		PV2103 (OSHA)	5.2.90
Chlorosarin	1445-76-7	TO-17 (EPA ORD)	5.2.49
Chlorosoman	7040-57-5	EPA/600/R-16/115	5.2.56
2-Chlorovinylarsonic acid (CVAOA) 2-Chlorovinylarsonous acid (CVAA)	64038-44-4 85090-33-1	**Analyze as CVAA and CVAOA**	
		EPA/600/R-15/258	5.2.54
		Analyze as total arsenic	
		200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Chlorpyrifos	2921-88-2	525.2 (EPA OW)	5.2.8
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55

Analyte	CAS RN	Method	Section
Chlorpyrifos oxon	5598-15-2	540 (EPA OW)	5.2.12
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Crimidine	535-89-7	EPA/600/R-16/114	5.2.55
Cyanide, Amenable to chlorination	NA	RLAB Method 3135.2I (EPA Region 7)	5.2.42
		4500-CN G (SM)	5.2.102
Cyanide, Total	57-12-5	335.4 (EPA OW)	5.2.5
		ISM02.3 CN (EPA CLP)	5.2.41
		6010 (NIOSH)	5.2.73
Cyanogen chloride	506-77-4	TO-15 (EPA ORD)	5.2.48
		Encyclopedia of Anal. Chem. (2006) DOI:10.1002/9780470027318.a0809	5.2.108
Cyclohexyl sarin (GF)	329-99-7	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115	5.2.56
1,2-Dichloroethane	107-06-2	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
Dichlorvos	62-73-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Dicrotophos	141-66-2	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Diesel range organics	NA	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36
Diisopropyl methylphosphonate (DIMP)	1445-75-6	538 (EPA OW)	5.2.11
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100
Dimethylphosphite	868-85-9	EPA/600/R-16/114	5.2.55
		TO-10A (EPA ORD)	5.2.47
Dimethylphosphoramidic acid	33876-51-6	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100

Analyte	CAS RN	Method	Section
Diphacinone	82-66-6	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		D7644-16 (ASTM)	5.2.96
Disulfoton Disulfoton sulfone oxon Disulfoton sulfoxide Disulfoton sulfoxide oxon	298-04-4 2496-91-5 2497-07-6 2496-92-6	525.2 (EPA OW)	5.2.8
		EPA/600/R-16/114	5.2.55
		5600 (NIOSH)	5.2.69
1,4-Dithiane	505-29-3	EPA/600/R-16/114	5.2.55
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-15/097	5.2.53
Ethyl methylphosphonic acid (EMPA)	1832-53-7	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100
Ethyldichloroarsine (ED)	598-14-1	3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		8270E (EPA SW-846)	5.2.35
		TO-15 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.80
N-Ethyldiethanolamine (EDEA)	139-87-7	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA / CDC)	5.2.50
		3509 (NIOSH)	5.2.67
		D7599-16 (ASTM)	5.2.95
Ethylene oxide	75-21-8	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
Fenamiphos	22224-92-6	525.2 (EPA OW)	5.2.8
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Fentanyl	437-38-7	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		L-A-309 Rev. 0 (EPA SOP)	5.2.60
		L-A-310 Rev. 1 (EPA SOP)	5.2.61
		J. Chromatogr. A (2011) 1218: 1620–1649	5.2.116

Analyte	CAS RN	Method	Section
Fluoride	16984-48-8	300.1, Rev 1.0 (EPA OW)	5.2.4
Fluoroacetamide	640-19-7	J. Chromatogr. B (2008) 876(1): 103–108	5.2.118
Fluoroacetic acid and fluoroacetate salts	NA	EPA/600/R-18/056	5.2.58
		S301-1 (NIOSH)	5.2.83
		J. Chromatogr. A (2007) 1139: 271–278	5.2.112
2-Fluoroethanol	371-62-0	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		2513 (NIOSH)	5.2.66
Fluorosilicic acid (analyze as fluoride)	16961-83-4	300.1, Rev 1.0 (EPA OW)	5.2.4
Formaldehyde	50-00-0	556.1 (EPA OW)	5.2.15
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8315A (EPA SW-846)	5.2.37
		2016 (NIOSH)	5.2.65
Gasoline range organics	NA	3570 (EPA SW-846)	5.2.24
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
		Analyst (2001) 126: 1689–1693	5.2.107
Hydrogen bromide Hydrogen chloride	10035-10-6 7647-01-0	7907 (NIOSH)	5.2.79
Hydrogen cyanide	74-90-8	6010 (NIOSH)	5.2.73
Hydrogen fluoride	7664-39-3	7906 (NIOSH)	5.2.78
Hydrogen sulfide	2148878	6013 (NIOSH)	5.2.74
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100
Kerosene	64742-81-0	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36

Analyte	CAS RN	Method	Section
Lead arsenate (analyze as total arsenic)	7645-25-2	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] Lewisite oxide	541-25-3 40334-69-8 40334-70-1 1306-02-1	**Analyze as lewisite I, 2, 3 or lewisite oxide**	
		EPA/600/R-15/258	5.2.54
		Analyze as total arsenic	
		200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Mercuric chloride (analyze as total mercury)	7487-94-7	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		9102 (NIOSH)	5.2.80
Mercury, Total	7439-97-6	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		IO-5 (EPA ORD)	5.2.46
		9102 (NIOSH)	5.2.80
Methamidophos	10265-92-6	538 (EPA OW)	5.2.11
		J. Env. Sci. Health (2014) 49: 23–34	5.2.113
		J. Chromatogr. A (2007) 1154(1): 3–25	5.2.114
Methomyl	16752-77-5	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.70
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		IO-5 (EPA ORD)	5.2.46
		9102 (NIOSH)	5.2.80

Analyte	CAS RN	Method	Section
Methyl acrylonitrile	126-98-7	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.24
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		8290A Appendix A (EPA SW-846)	5.2.36
		PV2004 (OSHA)	5.2.89
3-Methyl fentanyl	42045-87-4	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		L-A-309 Rev. 0 (EPA SOP)	5.2.60
		L-A-310 Rev. 1 (EPA SOP)	5.2.61
		J. Chromatogr. B (2014) 962: 52–58	5.2.119
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	EPA/600/R-18/056	5.2.58
		S301-1 (NIOSH)	5.2.83
		J. Chromatogr. A (2007) 1139: 271–278	5.2.112
Methyl hydrazine	60-34-4	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		3510 (NIOSH)	5.2.68
		J. Chromatogr. (1993) 617: 157–162	5.2.110
Methyl isocyanate	624-83-9	OSHA 54	5.2.85
Methyl paraoxon	950-35-6	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
Methyl parathion	298-00-0	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Methylamine	74-89-5	OSHA 40	5.2.84
N-Methyldiethanolamine (MDEA)	105-59-9	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA / CDC)	5.2.50
		3509 (NIOSH)	5.2.67
		D7599-16 (ASTM)	5.2.95
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115	5.2.56
Methylphosphonic acid (MPA)	993-13-5	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100
Mevinphos	7786-34-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55

Analyte	CAS RN	Method	Section
Monocrotophos	6923-22-4	3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8270E (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)-ethylamine] Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)-methylamine] Mustard, nitrogen (HN-3) [tris(2-chloroethyl)-amine]	538-07-8 51-75-2 555-77-1	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653	5.2.51
Mustard, sulfur / Mustard gas (HD)	505-60-2	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115	5.2.56
Nicotine compounds	54-11-5	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114	5.2.55
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Osmium tetroxide (analyze as total osmium)	20816-12-0	3015A (EPA SW-846)	5.2.16
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
Oxamyl	23135-22-0	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.70
		D7645-16 (ASTM)	5.2.97
Paraoxon	311-45-5	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Paraquat	4685-14-7	549.2 (EPA OW)	5.2.13
		J. Chromatogr. A (2008) 1196-97: 110–116	5.2.115

Analyte	CAS RN	Method	Section
Parathion	56-38-2	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Pentaerythritol tetranitrate (PETN)	78-11-5	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Phencyclidine	77-10-1	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
		9106 (NIOSH)	5.2.81
		9109 (NIOSH)	5.2.82
Phorate	298-02-2	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Phorate sulfone Phorate sulfone oxon	2588-04-7 2588-06-9	540 (EPA OW)	5.2.12
		TO-10A (EPA ORD)	5.2.47
Phorate sulfoxide Phorate sulfoxide oxon	2588-03-6 2588-05-8	EPA/600/R-16/114	5.2.55
Phosgene	75-44-5	OSHA 61	5.2.86
Phosphamidon	13171-21-6	525.3 (EPA OW)	5.2.9
		3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114	5.2.55
		TO-10A (EPA ORD)	5.2.47
Phosphine	7803-51-2	6002 (NIOSH)	5.2.72
Phosphorus trichloride	7719-12-2	6402 (NIOSH)	5.2.76
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224	5.2.52
		D7597-16 (ASTM)	5.2.93
		E2866-12 (ASTM)	5.2.100
Propylene oxide	75-56-9	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		1612 (NIOSH)	5.2.64
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653	5.2.51
Sarin (GB)	107-44-8	TO-17 (EPA ORD)	5.2.49
Soman (GD)	96-64-0	EPA/600/R-16/115	5.2.56

Analyte	CAS RN	Method	Section
Sodium arsenite (analyze as total arsenic)	7784-46-5	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Sodium azide (analyze as azide ion)	26628-22-8	300.1, Rev 1.0 (EPA OW)	5.2.4
		ID-211 (OSHA)	5.2.87
		J. Forensic Sci. (1998) 43(1): 200–202	5.2.120
Strychnine	57-24-9	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114	5.2.55
Tabun (GA)	77-81-6	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653	5.2.51
Tetraethyl pyrophosphate (TEPP)	107-49-3	3511 (EPA SW-846)	5.2.19
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Tetramethylenedisulfotetramine (TETS)	80-12-6	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114	5.2.55
Thallium sulfate (analyze as total thallium)	10031-59-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80
Thiodiglycol (TDG)	111-48-8	TO-10A (EPA ORD)	5.2.47
		D7598-16 (ASTM)	5.2.94
		E2787-11 (ASTM)	5.2.98
		E2838-11 (ASTM)	5.2.99

Analyte	CAS RN	Method	Section
Thiofanox	39196-18-4	538 (EPA OW)	5.2.11
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		5601 (NIOSH)	5.2.70
		D7645-16 (ASTM)	5.2.97
1,4-Thioxane	15980-15-1	EPA/600/R-16/114	5.2.55
Titanium tetrachloride (analyze as total titanium)	7550-45-0	3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
Triethanolamine (TEA)	102-71-6	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA / CDC)	5.2.50
		3509 (NIOSH)	5.2.67
		D7599-16 (ASTM)	5.2.95
Trimethyl phosphite	121-45-9	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8270E (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7	8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.80

Analyte	CAS RN	Method	Section
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester] VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	21738-25-0 78-53-5	TO-17 (EPA ORD)	5.2.49
VM [phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester] VX [O-ethyl-S-(2-diisopropylaminoethyl) methyl-phosphonothiolate]	21770-86-5 50782-69-9	EPA/600/R-16/116	5.2.57
White phosphorus	12185-10-3	3570 (EPA SW-846)	5.2.24
		7580 (EPA SW-846)	5.2.32
		8290A Appendix A (EPA SW-846)	5.2.36
		7905 (NIOSH)	5.2.77
The following analytes should be prepared and/or analyzed by the following methods only if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation / determinative techniques identified for these analytes in Appendix A.			
Allyl alcohol	107-18-6	TO-10A (EPA ORD)	5.2.47
3-Chloro-1,2-propanediol	96-24-2	TO-15 (EPA ORD)	5.2.48
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	TO-15 (EPA ORD)	5.2.48
Diisopropyl methylphosphonate (DIMP)	1445-75-6	TO-15 (EPA ORD)	5.2.48
Mercuric chloride (analyze as total mercury)	7487-94-7	7470A (EPA SW-846)	5.2.29
Mercury, Total	7439-97-6	7471B (EPA SW-846)	5.2.30
Methamidophos	10265-92-6	5600 (NIOSH)	5.2.69
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	7470A (EPA SW-846)	5.2.29
		7471B (EPA SW-846)	5.2.30
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-15 (EPA ORD)	5.2.48
Sarin (GB) Soman (GD)	107-44-8 96-64-0	TO-15 (EPA ORD)	5.2.48
1,4-Thioxane	15980-15-1	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34

Method summaries are listed in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), and literature references. Methods are listed in numerical order under each publisher. Where available, a direct link to the full text of the method is provided in the method summary. For additional information on preparation procedures and methods available through consensus standards organizations, please use the contact information provided in **Table 5-2**.

Table 5-2. Sources of Chemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
EPA Contract Laboratory Program (CLP) Methods	EPA, CLP	https://www.epa.gov/clp
EPA Office of Water (OW) Methods	EPA OW	https://www.epa.gov/dwanalyticalmethods
EPA Solid Waste (SW)-846 Methods	EPA Office of Land and Emergency Management (OLEM)	https://www.epa.gov/hw-sw846/sw-846-compendium
EPA Office of Research and Development (ORD) Methods	EPA ORD	https://www.epa.gov/aboutepa/about-office-research-and-development-ord
EPA Air Toxics Methods	EPA Office of Air and Radiation (OAR)	https://www.epa.gov/amtic/air-monitoring-methods
EPA Analytical Protocols and Standard Operating Procedures	EPA Center for Environmental Security and Emergency Response (CESER) [formerly EPA National Homeland Security Research Center (NHSRC)]	https://www.epa.gov/homeland-security-research/forms/contact-us-about-homeland-security-research
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov/dts/sltc/methods/index.html
NIOSH Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater (SM), 23rd Edition, 2017*	American Public Health Association (APHA)	http://www.standardmethods.org
*Annual Book of ASTM Standards**	ASTM International	http://www.astm.org
International Organization for Standardization (ISO) Methods*	ISO	http://www.iso.org
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
Analyst*	Royal Society of Chemistry	http://www.rsc.org/Publishing/Journals/AN/
Journal of Chromatography A and B*	Elsevier Science Publishers	http://www.journals.elsevier.com/journal-of-chromatography-a/
Journal of Forensic Sciences*	ASTM International	https://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/index.html
Journal of Environmental Science Health	Taylor & Francis Online	https://www.tandfonline.com/toc/lesb20/current
Encyclopedia of Analytical Chemistry*	Wiley	https://onlinelibrary.wiley.com/doi/book/10.1002/9780470027318
European Journal of Lipid Science and Technology*	Wiley	https://www.wiley-vch.de/en/shop/journals/134
EPA WCIT	EPA OW Water Security Division (WSD)	https://www.epa.gov/waterdata/water-contaminant-information-tool-wcit
Analytical Chemistry*	American Chemical Society(ACS)	http://pubs.acs.org/journal/ancham

* Subscription and/or purchase required.

5.1.2 General QC Guidelines for Chemical Methods

Having analytical data of appropriate quality requires that laboratories: (1) conduct the necessary QC activities to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC, including corrective actions.[8] In addition to the laboratories being capable of generating accurate and precise data during site remediation, they must be able to deliver results in a timely and efficient manner. Therefore, laboratories must be prepared with calibrated instruments, the proper standards, standard analytical procedures, standard operating procedures, and qualified and trained staff. Moreover, laboratories also must be capable of providing rapid turnaround of sample analyses and data reporting.

The level or amount of QC needed during sample analysis and reporting depends on the intended purpose of the data that are generated (e.g., the decision(s) to be made). The specific needs for data generation should be identified. QC requirements and DQOs should be derived based on those needs, and should be applied consistently across laboratories when multiple laboratories are used. For almost all of the chemical warfare agents (CWAs), most laboratories will not have access to analytical standards for calibration and QC. Use of these agents is strictly controlled by the Department of Defense (DoD) and access is limited. For information regarding laboratory analysis of samples containing CWAs or laboratory requirements to possess and use ultra-dilute agent standards, please use the contact information provided on the Environmental Response Laboratory Network (ERLN) website at: https://www.epa.gov/emergency-response/environmental-response-laboratory-network.

A minimum set of analytical QC procedures should be planned, documented and conducted for all chemical testing. Some method-specific QC requirements are described in many of the individual methods that are cited in this document and will be referenced in any analytical protocols developed to address specific analytes and sample types of concern. Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine if any additional QC might be needed. Analytical QC requirements generally consist of analysis of laboratory control samples to document whether the analytical system is in control; matrix spikes to identify and quantify measurement system accuracy for the media of concern and, at the levels of concern, various blanks as a measure of freedom from contamination; as well as matrix spike duplicates or sample replicates to assess data precision.

In general, for measurement of chemical analytes, appropriate QC includes an initial demonstration of measurement system capability, as well as ongoing analysis of standards and other samples to ensure the continued reliability of the analytical results. Examples of appropriate QC include:

- Initial demonstration that the measurement system is operating properly
  - Initial calibration
  - Laboratory blanks
  - Initial precision and recovery (IPR) samples
- Demonstration of analytical method suitability for intended use
  - Detection and quantitation limits
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte / matrix / level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)
- Demonstration of continued analytical method reliability
  - Analytical sample duplicates/replicates
  - Ongoing precision and recovery (OPR) samples at levels of concern

[8] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality.

- Surrogate spikes (where appropriate)
- Continuing calibration verification
- Method blanks

QC tests should be consistent with EPA's Good Laboratory Practice Standards (https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program) and be run as frequently as necessary to ensure the reliability of analytical results. Additional guidance can be found at: https://www.epa.gov/quality; in Chapter 1 of EPA SW-846 "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods" (https://www.epa.gov/sites/production/files/2015-10/documents/chap1_1.pdf); and in EPA's 2005 "Manual for the Certification of Laboratories Analyzing Drinking Water" (EPA 815-R-05-004) (https://nepis.epa.gov/Exe/ZyPDF.cgi/30006MXP.PDF?Dockey=30006MXP.PDF). As with the identification of needed QC samples, the frequency of QC sampling should be established based on an evaluation of DQOs. The type and frequency of QC tests can be refined over time.

Ensuring data quality also requires that laboratory results are properly assessed and documented. The results of the data quality assessment are included within the data report when transmitted to decision makers. This evaluation is as important as the data for ensuring informed and effective decisions. While some degree of data evaluation is necessary in order to be able to confirm data quality, 100% verification and/or validation is neither necessary nor conducive to efficient decision making in emergency situations. The level of such reviews should be determined based on the specific situation being assessed and on the corresponding DQOs. In every case, the levels of QC and data review necessary to support decision making should be determined as much in advance of data collection as possible.

Please note: The type and quantity of appropriate quality assurance (QA) and QC procedures that will be required are incident-specific and should be included in incident-specific documents (e.g., Quality Assurance Project Plan [QAPP], Sampling and Analysis Plan [SAP], laboratory Statement of Work [SOW], analytical methods). This documentation and/or Incident Command should be consulted regarding appropriate QA and QC procedures prior to sample analysis.

5.1.3 Safety and Waste Management

It is imperative that safety precautions are used during collection, processing and analysis of environmental samples. Laboratories should have a documented health and safety plan for handling samples that may contain the target chemical, biological and/or radiological (CBR) contaminants. Laboratory staff should be trained in, and need to implement, the safety procedures included in the plan. In addition, many of the methods summarized or cited in Section 5.2 contain some specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents.

These methods also provide information regarding waste management. Other resources that can be consulted for additional information include the following:

- Centers for Disease Control and Prevention (CDC) – Title 42 of the Code of Federal Regulations part 72 (42 CFR 72). Interstate Shipment of Etiologic Agents
- CDC – 42 CFR part 73. Select Agents and Toxins
- Department of Transportation (DOT) – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training Requirements
- EPA – 40 CFR part 260. Hazardous Waste Management System: General
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program

- OSHA – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response

Please note that the Electronic Code of Federal Regulations (e-CFR) is available at: http://www.ecfr.gov/cgi-bin/ECFR?page=browse.

5.2 Method Summaries

Summaries for the analytical methods listed in Appendix A are provided in Sections 5.2.1 through 5.2.120. These sections contain summary information extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix A to which the method applies, a brief description of the analytical method, and a link to, or source for, obtaining a full version of the method. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 5.1.1) can be found in Appendix A. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

5.2.1 EPA Method 200.7: Determination of Metals and Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

Method Developed for: Determination of metals in aqueous and solid samples
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Method detection limits (MDLs) in aqueous samples are reported for arsenic (8 µg/L), vanadium (3 µg/L) and thallium (1 µg/L).

Description of Method: This method will determine metal-containing compounds only as the total metal (e.g., total arsenic) in aqueous samples. An aliquot of a well-mixed, homogeneous sample is accurately weighed or measured for sample processing. For total recoverable analysis of a sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight

prior to analysis. For determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 nephelometric turbidity units (NTU), the sample is made ready for analysis by the addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The prepared sample is analyzed using ICP-AES. Specific analytes targeted by Method 200.7 are listed in Section 1.1 of the method.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: Martin, T.D., Brockhoff, C.A., Creed, J.T. and EMMC Methods Work Group. 1994. "Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4.4. Cincinnati, OH: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.7.pdf

5.2.2 EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Inductively coupled plasma-mass spectrometry (ICP-MS)

Method Developed for: Dissolved and total elements in ground water, surface water, drinking water, wastewater, sludges and soils
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for arsenic, thallium and vanadium in aqueous samples are reported as 1.4, 0.3 and 2.5 µg/L, respectively (in scanning mode) and 0.4, 0.02 and 0.9 µg/L, respectively (in selected ion monitoring (SIM) mode). The recommended calibration range is 10–200 µg/L (scanning mode) and may be lower depending on the sensitivity of the instrument.

Description of Method: This method will determine metal-containing compounds only as the total metal (e.g., total arsenic). An aliquot of a well-mixed, homogeneous sample is accurately weighed or measured for sample processing. For total recoverable analysis of a sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. For determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 NTU, the sample is made ready for analysis by the addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The prepared sample is analyzed using ICP-MS. Specific analytes targeted by Method 200.8 are listed in Section 1.1 of the method.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: Creed, J.T., Brockhoff, C.A. and Martin, T.D. 1994. "Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry," Revision 5.4. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf

5.2.3 EPA Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry

Analyte(s)	CAS RN
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Cold vapor atomic absorption

Method Developed for: Mercury in surface waters. It may be applicable to saline waters, wastewaters, effluents, and domestic sewages providing potential interferences are not present.
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Applicable concentration range is 0.2–10.0 µg Hg/L. The detection limit for this method is 0.2 µg Hg/L.

Description of Method: This method will determine mercuric chloride and methoxyethylmercuric acetate as total mercury. If dissolved mercury is targeted, the sample is filtered prior to acidification. To detect total mercury (inorganic and organic mercury), the sample is treated with potassium permanganate and potassium persulfate to oxidize organic mercury compounds prior to analysis. Inorganic mercury is reduced to the elemental state (using stannous chloride) and aerated from solution. The mercury vapor passes through a cell positioned in the light path of a cold vapor atomic absorption spectrophotometer. The concentration of mercury is measured using the spectrophotometer.

Special Considerations: If problems occur during analysis of aqueous samples other than drinking water, refer to Method 7470A (EPA SW-846).

Source: O'Dell, J.W., Potter, B.B., Lobring, L.B. and Martin, T.D. 1994. "Method 245.1: Determination

of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry," Revision 3.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-245.1.pdf

5.2.4 EPA Method 300.1, Revision 1.0: Determination of Inorganic Anions in Drinking Water by Ion Chromatography

Analyte(s)	CAS RN
Fluoride	16984-48-8
Fluorosilicic acid (analyze as fluoride)	16961-83-4
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: For fluoride and fluorosilicic acid, use direct injection. For sodium azide in water and solid samples, use water extraction, filtration and acidification steps from the Journal of Forensic Science, 1998. 43(1): 200-202 (Section 5.2.120).
Determinative Technique: Ion chromatography (IC) with conductivity detection

Method Developed for: Inorganic anions in reagent water, surface water, ground water and finished drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples for fluoride and fluorosilicic acid (as fluoride). It also has been selected for analysis of prepared solid samples for sodium azide (as azide ion). See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoride in reagent water is 0.009 mg/L. The MDL varies depending upon the nature of the sample and the specific instrumentation employed. The estimated calibration range should not extend more than 2 orders of magnitude in concentration over the expected concentration range of the samples.

Description of Method: This method will determine fluoride ion, fluorosilicic acids as fluoride ion, and sodium azide as azide ion. It was developed for analysis of aqueous samples, and can be adapted for analysis of sodium azide in solid and air samples when appropriate sample preparation techniques have been applied (see Appendix A). A small volume of a water sample (10 µL or 50 µL) is introduced into an ion chromatograph. The volume selected depends on the concentration of fluoride or azide ion in the sample. The anions of interest are separated and measured, using a system comprising a guard column, analytical column, suppressor device and conductivity detector. The separator columns and guard columns, as well as eluent conditions, are identical. To achieve comparable detection limits, an ion chromatographic system must use suppressed conductivity detection, be properly maintained, and be capable of yielding a baseline with no more than 5 nano siemens (nS) noise/drift per minute of monitored response over the background conductivity.

Special Considerations: For sodium azide, if analyses are problematic, refer to the column manufacturer for alternate conditions.

Source: Hautman, D.P. and Munch, D.J. 1997. "Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography," Revision 1.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-300.1.pdf

5.2.5 EPA Method 335.4: Determination of Total Cyanide by Semi-Automated Colorimetry

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Reflux-distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Cyanide in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address total cyanide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The applicable range is 5–500 µg/L.

Description of Method: Cyanide is released from cyanide complexes as hydrocyanic acid by manual reflux-distillation, and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is converted to cyanogen chloride by reaction with chloramine-T, which subsequently reacts with pyridine and barbituric acid to give a red-colored complex.

Special Considerations: Interferences include aldehydes, nitrate-nitrite and oxidizing agents, such as chlorine, thiocyanate, thiosulfate and sulfide. These interferences can be eliminated or reduced by distillation.

Source: U.S. EPA. 1993. "Method 335.4: Determination of Total Cyanide by Semi-automated Colorimetry," Revision 1.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-335.4.pdf

5.2.6 EPA Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Ammonia in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range for ammonia is 0.01–2.0 mg/L.

Description of Method: This method identifies and determines the concentration of ammonia in drinking water samples by spectrophotometry. Samples are buffered at a pH of 9.5 with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and are distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured spectrophotometrically.

Special Considerations: Reduced volume distillation techniques, such as midi-distillation or micro-

distillation, can be used in place of traditional macro-distillation techniques.

Source: U.S. EPA. 1993. "Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)," Revision 2.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-350.1.pdf

5.2.7 EPA Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography / Mass Spectrometry

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Carbon disulfide	75-15-0
1,2-Dichloroethane	107-06-2
Methyl acrylonitrile	126-98-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-MS

Method Developed for: Purgeable volatile organic compounds (VOCs) in surface water, ground water and drinking water in any stage of treatment
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address carbon disulfide and 1,2-dichloroethane, and preparation and analysis of drinking and non-drinking water samples to address acrylonitrile and methyl acrylonitrile. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports detection levels for acrylonitrile, carbon disulfide, 1,2-dichloroethane and methyl acrylonitrile in reagent water of 0.22, 0.093, 0.02 and 0.11 µg/L, respectively. The applicable concentration range of this method is primarily column and matrix dependent, and is approximately 0.02–200 µg/L when a wide-bore thick-film capillary column is used. Narrow-bore thin-film columns may have a lower capacity, which limits the range to approximately 0.02–20 µg/L.

Description of Method: VOCs and surrogates with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through the sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and backflushed with helium to desorb the trapped sample components into a capillary gas chromatography (GC) column interfaced to a mass spectrometer (MS). The column is temperature programmed to facilitate the separation of the method analytes, which are then detected with the MS. Specific analytes targeted by Method 524.2 are listed in Section 1.1 of the method.

Special Considerations: The more recent versions of this method (Methods 524.3 or 524.4) may be used in place of Method 524.2, provided the laboratory has the necessary equipment (e.g., cryogenic auto samplers).

Source: Eichelberger, J.W., Munch, J.W. and Bellar, T.A. 1995. "Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 4.1. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-524.2.pdf

Additional Resources:

Prakash, A.D., Zaffiro, A.D., Zimmerman, M., Munch, D.J. and Pepich, B.V. 2009. "Method 524.3:

Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA 815-B-09-009. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J75C.PDF?Dockey=P100J75C.PDF

U.S. EPA. 2013. "Method 524.4: Measurement of Purgeable Organic Compounds in Water by Gas Chromatography/Mass Spectrometry," Revision 1. Cincinnati, OH: U.S. EPA. EPA 815-R-13-002. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7EE.PDF?Dockey=P100J7EE.PDF

5.2.8 EPA Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry

Analyte(s)	CAS RN
Chlorpyrifos	2921-88-2
Dichlorvos	62-73-7
Disulfoton*	298-04-4
Disulfoton sulfone oxon*	2496-91-5
Disulfoton sulfoxide*	2497-07-6
Disulfoton sulfoxide oxon*	2496-92-6
Fenamiphos	22224-92-6
Mevinphos	7786-34-7

* If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/article/pii/S0043135408004995

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Liquid-solid extraction (LSE) or solid-phase extraction (SPE)
Determinative Technique: GC-MS

Method Developed for: Organic compounds in finished drinking water, source water or drinking water in any treatment stage
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. ***Note***:

- EPA/600/R-16/114 (Section 5.2.55) has been selected for preparation and analysis of non-drinking water samples to address chlorpyrifos and fenamiphos.
- SW-846 Method 3535A (Section 5.2.21) and Method 8270E (Section 5.2.35) have been selected for preparation and analysis of non-drinking water samples to address dichlorvos and mevinphos.

See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The applicable concentration range for most analytes is 0.1–10 µg/L.

Description of Method: Organic compounds, internal standards and surrogates are extracted from a water sample by passing 1 L of sample through a cartridge or disk containing a solid matrix with chemically bonded C_{18} organic phase (LSE or SPE). The organic compounds are eluted from the LSE (SPE) cartridge or disk with small quantities of ethyl acetate followed by methylene chloride. The resulting extract is concentrated further by evaporation of some of the solvent. Sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high resolution fused silica capillary column of a GC-MS system. Specific analytes targeted by Method 525.2 are listed in Section 1.1 of the method.

Special Considerations: Refer to the footnote provided in the analyte table above for special considerations that should be applied when measuring specific analytes. SPE using C_{18} resin may not

work for certain compounds having high water solubility. In these cases, other sample preparation techniques or different SPE resins may be required. The more recent version of this method (Method 525.3) may be used in place of Method 525.2, provided the laboratory has the necessary equipment and expertise.

Source: Munch, J.W. 1995. "Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry," Revision 2.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-525.2.pdf

Additional Resource: Munch, J.W., Grimmett, P.E., Munch, D.J., Wendelken, S.C., Domino, M.M., Zaffiro, A.D. and Zimmerman, M.L. 2012. "Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-12/010. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=241188

5.2.9 EPA Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Phosphamidon	13171-21-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SPE
Determinative Technique: GC-MS

Method Developed for: Semivolatile organic compounds in drinking water.
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address phosphamidon. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The applicable concentration range for most analytes is 0.1–10 µg/L.

Description of Method: A 1-L sample is fortified with surrogates and passed through an SPE apparatus. Phosphamidon and surrogates are eluted from the solid phase with a small amount of two or more organic solvents. The solvent extract is dried by passing it through a column of anhydrous sodium sulfate, concentrated by nitrogen gas blow-down, and adjusted to a 1-mL volume with ethyl acetate after adding internal standards. A splitless injection is made into a GC equipped with a high-resolution fused silica capillary column interfaced to an MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical GC-MS conditions. The GC-MS can be operated in the full scan, SIM, or selected ion storage (SIS) mode. Analyte concentrations are calculated using their integrated peak area and the internal standard technique.

Special Considerations: Phosphamidon was observed to exhibit matrix induced chromatographic response enhancement during method development, determined by comparing the peak area response of a standard prepared in solvent compared to a matrix-matched standard, both at a concentration of 0.2 ng/µL. The method includes use of matrix-matched standards as an option to evaluate matrix interferences. If the peak area of a matrix-matched standard is ≥ 130% of the of the peak area produced by the solvent-prepared standard, matrix enhancement is likely to be present.

Source: Munch, J.W., Grimmett, P.E., Munch, D.J., Wendelken, S.C., Domino, M.M., Zaffiro, A.D. and Zimmerman, M.L. 2012. "Method 525.3 Determination of Semivolatile Organic Chemicals in

Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/Mass Spectrometry (GC/MS),” Version 1.0. Cincinnati, OH: U.S. EPA. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=241188

5.2.10 EPA Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection
Determinative Technique: High-performance liquid chromatography (HPLC)-fluorescence (FL)

Method Developed for: N-methylcarbamoyloximes and N-methylcarbamates in finished drinking water
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above. It has also been selected for preparation and analysis of non-drinking water samples to address methomyl. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits range from 0.026 to 0.115 µg/L. The concentration range for target analytes in this method was evaluated between 0.2 µg/L and 10 µg/L.

Description of Method: An aliquot of sample is measured in a volumetric flask. Samples are preserved, spiked with appropriate surrogates and then filtered. Analytes are chromatographically separated by injecting a sample aliquot (up to 1000 µL) into a HPLC system equipped with a reverse phase (C_{18}) column. After elution from the column, the analytes are hydrolyzed in a post column reaction to form methylamine, which is in turn reacted to form a fluorescent isoindole that is detected by an FL detector. Analytes also are quantitated using the external standard technique.

Source: Bassett, S.C., Wendelken, S.C., Pepich, B.V., Munch, D.J. and Henry, L. 2001. “Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization,” Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/815/B-01/002. https://www.epa.gov/sites/production/files/2015-06/documents/epa-531.2.pdf

5.2.11 EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)

Analyte(s)	CAS RN
Acephate	30560-19-1
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Methamidophos	10265-92-6
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and/or analyte determination and measurement

Sample Preparation Technique: Direct injection
Determinative Technique: Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS)

Method Developed for: Acephate, DIMP, methamidophos and thiofanox in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above, preparation and analysis of non-drinking water samples to address acephate and methamidophos, and analysis of prepared solid samples to address acephate and methamidophos. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDLs for acephate, DIMP, methamidophos and thiofanox in reagent water were calculated to be 0.019, 0.014, 0.017 and 0.090 µg/L, respectively. The Lowest Concentration Minimum Reporting Levels (LCMRLs) in reagent water were calculated to be 0.044, 0.022, 0.032 and 0.18 µg/L, respectively.

Description of Method: A 40-mL water sample is collected in a bottle containing sodium omadine and ammonium acetate. An aliquot of the sample is placed in an autosampler vial and internal standards are added. A 50-µL or larger injection is made into a liquid chromatograph (LC) equipped with a C_{18} column that is interfaced to an MS-MS operated in the electrospray ionization (ESI) mode. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by internal standard calibration using procedural standards.

Source: Shoemaker, J.A. 2009. "Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-09/149.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-538.pdf

5.2.12 EPA Method 540: Determination of Selected Organic Chemicals in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Chlorpyrifos oxon	5598-15-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SPE
Determinative Technique: LC-MS-MS

Method Developed for: Chlorpyrifos oxon, phorate sulfone and phorate sulfoxide in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Depending on the SPE cartridge used, detection limits were calculated to be 0.77 and 1.0 ng/L (chlorpyrifos oxon), 0.32 and 0.57 ng/L (phorate sulfone) and 0.46 and 0.70 ng/L (phorate sulfoxide). The LCMRLs in reagent water were calculated to be 2.0 and 2.7 ng/L (chlorpyrifos oxon), 0.86 and 1.0 ng/L (phorate sulfone) and 0.99 and 1.1 ng/L (phorate sulfoxide).

Description of Method: A 250-mL water sample is preserved with Trizma (Millipore Sigma, St. Louis, MO, or equivalent), 2-chloroacetamide and ascorbic acid. The sample is fortified with surrogates and passed through an SPE cartridge. Compounds are eluted from the solid phase with a small amount of methanol, and the extract is concentrated by evaporation with nitrogen in a heated water bath, internal standards are added, and the volume is adjusted to 1 mL with methanol. A 10-µL injection is made into an LC equipped with a C_{18} column that is interfaced to an MS-MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the internal standard technique.

Source: Shoemaker, J.A. and Tettenhorst, D.R. 2013. "Method 540: Determination of Selected Organic Chemicals in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-13/119.
https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P100H0Z5.txt

5.2.13 EPA Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection

Analyte(s)	CAS RN
Paraquat	4685-14-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: LSE or SPE
Determinative Technique: HPLC-ultraviolet (UV)

Method Developed for: Diquat and paraquat in drinking water sources and finished drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples to address paraquat. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for paraquat is 0.68 µg/L. The analytical range depends on the sample matrix and the instrumentation used.

Description of Method: A 250-mL sample is extracted using a C_8 LSE cartridge or a C_8 disk that has been specially prepared for the reversed-phase, ion-pair mode. The LSE disk or cartridge is eluted with acidic aqueous solvent to yield the eluate/extract. An ion-pair reagent is added to the eluate/extract. The concentrations of paraquat in the eluate/extract are measured using a HPLC system equipped with a UV absorbance detector. A photodiode array detector is used to provide simultaneous detection and confirmation of the method analytes.

Source: Munch, J.W. and Bashe, W.J. 1997. "Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection," Revision 1.0. Cincinnati, OH: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-549.2.pdf

5.2.14 EPA Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection

Analyte(s)	CAS RN
Chloropicrin	76-06-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-electron capture detector (ECD)

Method Developed for: Chlorination disinfection byproducts, chlorinated solvents and halogenated pesticides/herbicides in finished drinking water, drinking water during intermediate stages of treatment, and raw source water
Method Selected for: This method has been selected for preparation and analysis of water samples to address chloropicrin. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The estimated detection limit (EDL) using methyl *tert*-butyl ether (MTBE) and ammonium chloride-preserved reagent water on a 100% dimethylpolysiloxane (DB-1) column has been found to be 0.014 µg/L.

Description of Method: This is a GC-ECD method applicable to the determination of halogenated analytes in finished drinking water, drinking water during intermediate stages of treatment, and raw source water. A 50-mL sample aliquot is extracted with 3 mL of MTBE or 5 mL of pentane. Two µL of the extract is then injected into a GC equipped with a fused silica capillary column and linearized ECD for separation and analysis. This liquid/liquid extraction technique efficiently extracts a wide boiling range of non-polar and polar organic components of the sample. Thus, confirmation is quite important, particularly at lower analyte concentrations. A confirmatory column is suggested for this purpose.

Special Considerations: The presence of chloropicrin should be confirmed using either a secondary GC column or an MS.

Source: Munch, D.J. and Hautman, D.P. 1995. "Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection," Revision 1.0. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-551.1.pdf

5.2.15 EPA Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Liquid-liquid extraction with hexane
Determinative Technique: Fast gas chromatography with electron capture detection (FGC-ECD)

Method Developed for: Formaldehyde in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address formaldehyde. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: MDLs for formaldehyde in reagent water were calculated as 0.09 and 0.08 µg/L for primary and secondary columns, respectively. The applicable concentration range is approximately 5–40 µg/L.

Description of Method: A 20-mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 ºC for 2 hours with 15 mg of O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL of hexane. The extract is processed through an acidic wash step, and analyzed by FGC-ECD. The target analytes are identified and quantified by comparison to a procedural standard. Two

chromatographic peaks will be observed for many of the target analytes. Both (E) and (Z) isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. The (E) and (Z) isomers may not be chromatographically resolved in a few cases. Compounds with two carbonyl groups, such as glyoxal and methyl glyoxal, can produce even more isomers. Chromatographic peaks used for analyte identification are provided in Section 17, Table 1 and Figure 1 of the method.

Special Considerations: All results should be confirmed on a second, dissimilar capillary GC column.

Source: Wendelken, S.C., Pepich, B.V. and Munch, D.J. 1999. "Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography," Revision 1.0. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-556.1.pdf

5.2.16 EPA Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microwave assisted acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in water, mobility-procedure extracts, and wastes that contain suspended solids
Method Selected for: This method has been selected for preparation of non-drinking water samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; thallium sulfate as total thallium; osmium tetroxide as osmium; and ammonium

metavanadate and vanadium pentoxide as total vanadium. A 45-mL aliquot of a well-shaken, homogenized sample is transferred to a fluorocarbon polymer or quartz microwave vessel or vessel liner, equipped with a controlled pressure relief mechanism. 5 mL of concentrated nitric acid or 4 mL of concentrated nitric acid plus 1 mL of concentration hydrochloric acid are added to the vessel. The vessel is sealed, placed into the microwave system, and heated. The temperature of each sample should rise to 170 ± 5°C in approximately 10 minutes and remain at that temperature for 10 minutes, or for the remainder of the 20-minute digestion period. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume. Samples are analyzed for total arsenic, total osmium, total thallium, or total vanadium by SW-846 Method 6010D (Section 5.2.27) or 6020B (Section 5.2.28).

Special Considerations: Digestion of samples that contain organics may create high pressures due to the evolution of gaseous digestion products. This may cause venting of the vessels with potential loss of sample components and/or analytes. In these cases, a smaller sample size should be used, but the volume prior to addition of acid(s) should be adjusted to 45 mL with deionized water. Highly reactive samples may also require pre-digestion in a hood to minimize the danger of thermal runaway and excessively vigorous reactions. Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit the quantitation techniques when using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 2007. "Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf

5.2.17 EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in sediments, sludges, and soil samples
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; thallium sulfate as total thallium; and ammonium metavanadate and vanadium pentoxide as total vanadium. A 1-g to 2-g sample is digested with nitric acid and hydrogen peroxide. Sample volumes are reduced, then brought up to a final volume of 100 mL. Samples are analyzed for total arsenic, total osmium, total thallium, total titanium or total vanadium by Method 6010D (Section 5.2.27) or 6020B (Section 5.2.28).

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 1996. "Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils," Revision 2. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

5.2.18 EPA Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microwave assisted acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in sediments, sludges, soils, and oils
Method Selected for: This method has been selected for:

- Preparation of solid samples to be analyzed for the total arsenic component of arsenic trioxide arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite;
- Preparation of solid samples to be analyzed for the total thallium component of thallium sulfate;
- Preparation of solid samples to be analyzed for the total vanadium component of ammonium metavanadate and vanadium pentoxide;
- Preparation of solid and wipe samples to be analyzed for the total osmium component of osmium tetroxide; and
- Preparation of solid and wipe samples to be analyzed for the total titanium component of titanium tetrachloride.

NIOSH Method 9102 (see Section 5.2.80) should be used for the preparation of wipes to be analyzed for all other analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: A well-mixed sample (no more than 0.500 g for soils, sediments and sludges, and no more than 0.250 g for oil or oil contaminated soil) to the nearest 0.001 g is weighed into a fluorocarbon polymer or quartz microwave vessel or vessel liner equipped with a controlled pressure relief mechanism. 10 mL of concentrated nitric acid or 9 mL of concentrated nitric acid plus 3 mL of concentration hydrochloric acid are added to the vessel, and the vessel is sealed, placed into the microwave system, and heated. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume. Samples are analyzed for total arsenic, total osmium, total thallium, total titanium or total vanadium by Method 6010D or 6020B (SW-846).

Special Considerations: Digestion of samples that contain organics or carbonates can create high pressures due to the evolution of gaseous digestion products. This can cause venting of the vessels with potential loss of sample components and/or analytes. Samples that are highly reactive or contaminated might require dilution or pre-digestion in a hood to minimize the danger of thermal runaway and excessively vigorous reactions. Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit the quantitation techniques when using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: U.S. EPA. 2007. "Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

5.2.19 EPA Method 3511 (SW-846): Organic Compounds in Water by Microextraction

Analyte(s)	CAS RN
Tetraethyl pyrophosphate (TEPP)	107-49-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microextraction
Determinative Technique: GC-MS
Determinative Method: EPA SW-846 Method 8270E (Section 5.2.35)

Method Developed for: Organic compounds in aqueous samples
Method Selected for: This method has been selected for preparation of water samples to address TEPP.

See Appendix A for corresponding method usability tiers.

Description of Method: A measured volume of water sample, usually 35 mL, is placed into a 40-mL volatile organic analysis (VOA) vial. Surrogates (10 µg of each), 2 mL of methylene chloride, and 12 g of sodium chloride are added to the vial, and the vial is capped and shaken vigorously for 5 minutes or until the sodium chloride is completely dissolved. After the contents are allowed to settle (centrifuging if necessary), 1.5 mL of the lower (methylene chloride) layer is transferred to a 2-mL vial. The extract is then dried with sodium sulfate and a 1-mL aliquot is transferred to a GC vial. Samples are analyzed for TEPP by SW-846 Method 8270E (Section 5.2.35).

Source: U.S. EPA. 2014. "Method 3511 (SW-846): Organic Compounds in Water by Microextraction," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3511.pdf

5.2.20 EPA Method 3520C (SW-846): Continuous Liquid-Liquid Extraction

Analyte(s)	CAS RN
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Diesel range organics	NA
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Paraoxon	311-45-5
Parathion	56-38-2
Phosphamidon	13171-21-6

Analysis Purpose: Sample preparation
Sample Preparation Technique: Continuous liquid-liquid extraction (CLLE)
Determinative Technique: GC-flame ionization detector (FID) / GC-MS / LC-MS-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33), Method 8270E (Section 5.2.35), adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119), or adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.116). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in aqueous samples
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. ***Note***: Drinking water samples to be analyzed for phosphamidon should be prepared and analyzed using EPA Method 525.3 (Section 5.2.9). See Appendix A for corresponding method usability tiers.

Description of Method: This method is applicable to the isolation and concentration of water-insoluble and slightly soluble organics in preparation for a variety of chromatographic procedures. A measured volume of sample, usually 1 L, is placed into a continuous liquid-liquid extractor, adjusted, if necessary, to a specific pH and extracted with organic solvent for 18 to 24 hours. The extract is filtered through sodium sulfate to remove residual moisture, concentrated, and exchanged as necessary into a solvent compatible with the cleanup or determinative procedure used for analysis.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 1996. "Method 3520C (SW-846): Continuous Liquid-Liquid Extraction." Revision 3. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3520c.pdf

5.2.21 EPA Method 3535A (SW-846): Solid-Phase Extraction

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Ethyldichloroarsine (ED)	598-14-1
Fentanyl	437-38-7
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Paraoxon	311-45-5
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phorate	298-02-2
Phosphamidon	13171-21-6
Strychnine	57-24-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7

Analysis Purpose: Sample preparation
Sample Preparation Technique: SPE
Determinative Technique: GC-FID / GC-MS / LC-MS-MS / HPLC
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33), Method 8270E (Section 5.2.35), Method 8330B (Section 5.2.40), adapted from Analyst, 126:1689-1693 (Section 5.2.107), adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119), or adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.116). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in ground water, wastewater and Toxicity Characteristic Leaching Procedure (TCLP, Method 1311) leachates
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. ***Note***:

- EPA Method 525.2 (Section 5.2.8) has been selected for preparation and analysis of drinking water samples to address dichlorvos and mevinphos.
- EPA Method 525.3 (Section 5.2.9) has been selected for preparation and analysis of drinking water samples to address phosphamidon.

All other drinking water samples and all non-drinking water samples should be prepared using this method (SW-846 Method 3535A). See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a procedure for isolating target organic analytes from

samples using SPE media. Sample preparation procedures vary by analyte group. Following pH adjustment, a measured volume of sample is extracted by passing it through the SPE medium (disks or cartridges), which is held in an extraction device designed for vacuum filtration of the sample. Target analytes are eluted from the solid-phase media using an appropriate solvent which is collected in a receiving vessel. The resulting solvent extract is dried using sodium sulfate and concentrated, as needed.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 2007. "Method 3535A (SW-846): Solid-Phase Extraction (SPE)," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3535a.pdf

5.2.22 EPA Method 3541 (SW-846): Automated Soxhlet Extraction

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
Monocrotophos	6923-22-4
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Automated Soxhlet extraction
Determinative Technique: GC-FID / GC-MS / LC-MS-MS / HPLC-UV
Determinative Method: EPA SW-846 Methods 8015D (Section 5.2.33) and 8270E (Section 5.2.35), EPA/600/R-15/097 (Section 5.2.53), EPA/600/R-11/143 (Section 5.2.50), ASTM Methods D7644 (Section 5.2.96) and D7645 (Section 5.2.97), adapted from J. Chromatogr. 617: 157-162 (Section 5.2.110), adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.114), adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.116), or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soil, sediment, sludges and waste solids
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: Approximately 10 g of solid sample is mixed with an equal amount of anhydrous sodium sulfate and placed in an extraction thimble or between two plugs of glass wool. After adding the appropriate surrogate amount, the sample is extracted using an appropriate solvent in an automated Soxhlet extractor. The extract is dried with sodium sulfate to remove residual moisture,

concentrated and exchanged, as necessary, into a solvent compatible with the cleanup or determinative procedure for analysis.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 1994. "Method 3541 (SW-846): Automated Soxhlet Extraction," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3541.pdf

5.2.23 EPA Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
Monocrotophos	6923-22-4
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Pressurized fluid extraction (PFE)
Determinative Technique: GC-FID / GC-MS / LC-MS-MS / HPLC-UV
Determinative Method: EPA SW-846 Methods 8015D (Section 5.2.33) and 8270E (Section 5.2.35), EPA/600/R-15/097 (Section 5.2.53), EPA/600/R-11/143 (Section 5.2.50), ASTM Methods D7644 (Section 5.2.96) and D7645 (Section 5.2.97), adapted from J. Chromatogr. 617: 157-162 (Section 5.2.110), adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.117), adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.116), or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soils, clays, sediments, sludges and waste solids
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, and 50 to 5000 µg/kg of chlorinated herbicides.

Description of Method: Approximately 10 to 30 g of soil sample is prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. The extraction cell containing the sample is heated to the extraction temperature, pressurized with the appropriate solvent system, and

extracted for 5 minutes (or as recommended by the instrument manufacturer). The extract may be concentrated, if necessary, and exchanged into a solvent compatible with the cleanup or determinative step being employed.

Special Considerations: Sodium sulfate can cause clogging, and air-drying or pelletized diatomaceous earth may be preferred for drying samples. Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 2007. "Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3545a.pdf

5.2.24 EPA Method 3570 (SW-846): Microscale Solvent Extraction (MSE)

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carbofuran (Furadan)	1563-66-2
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl hydrazine	60-34-4
Monocrotophos	6923-22-4
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Pentaerythritol tetranitrate (PETN)	78-11-5
Thiofanox	39196-18-4
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microscale solvent extraction (MSE)
Determinative Technique: GC – nitrogen-phosphorus detector (NPD) / GC-FID / GC-MS / HPLC-UV / LC-MS-MS
Determinative Method: EPA SW-846 Methods 7580 (Section 5.2.32), 8015D (Section 5.2.33), 8260D (Section 5.2.34), 8270E (Section 5.2.35), 8315A (Section 5.2.37), 8316 (Section 5.2.38), 8318A (Section 5.2.39) and 8330B (Section 5.2.40); EPA/600/R-15/097 (Section 5.2.53); ASTM Methods D7644-16

(Section 5.2.96) and D7645-16 (Section 5.2.97); adapted from Analyst, 126:1689-1693 (Section 5.2.107); adapted from J. Chromatogr. 617: 157-162 (Section 5.2.110); adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.117); or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Extracting volatile, semivolatile and nonvolatile organic compounds from solids such as soils, sludges and wastes
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: Samples are prepared by shake extraction with an organic solvent in sealed extraction tubes. Careful manipulation of the sample, solvent, drying agent and spiking solutions minimizes loss of volatile compounds while maximizing extraction of volatile, semivolatile and nonvolatile compounds. Sample extracts are collected, dried, and concentrated using a modification of the Kuderna-Danish concentration method or other appropriate technique. By increasing the number of theoretical plates and reducing the distillation temperature, extracts are concentrated without loss of volatile constituents. Samples should be prepared one at a time to the point of solvent addition (i.e., do not pre-weigh a number of samples then add the solvent). Samples should be extracted as soon after collection as possible, and exposure to air before sample extraction minimized as much as possible.

Special Considerations: Method 3570 is not amenable for analysis of samples that have been preserved in the field using methanol.

Source: U.S. EPA. 2002. "Method 3570 (SW-846): Microscale Solvent Extraction (MSE)," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-3570.pdf

5.2.25 EPA Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Propylene oxide	75-56-9
The following analyte should be prepared by this method (and determined by the corresponding SW-846 Method 8260D) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-FID / GC-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33) or Method 8260D (Section 5.2.34). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: VOCs in aqueous and water miscible liquid samples
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. **Note**: For carbon disulfide and 1,2-dichloroethane, EPA Method 524.2

(Section 5.2.7) should be used for preparation of drinking water samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a purge-and-trap procedure for the analysis of VOCs in aqueous liquid samples and water miscible liquid samples. An inert gas is bubbled through a portion of the aqueous liquid sample at ambient temperature, and the volatile components are transferred from the aqueous liquid phase to the vapor phase. The vapor is swept through a sorbent column where the volatile components are adsorbed. After purging is completed, the sorbent column is heated and backflushed with inert gas to desorb the components onto a GC column.

Special Considerations: Heated purge may be required for poor-purging analytes.

Source: U.S. EPA. 2003. "Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples," Revision 3. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-5030c.pdf

5.2.26 EPA Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Methyl acrylonitrile	126-98-7
Propylene oxide	75-56-9
The following analyte should be prepared by this method (and determined by the corresponding SW-846 Method 8260D) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-FID / GC-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33) or Method 8260D (Section 5.2.34). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: VOCs in solid materials (e.g., soils, sediments and solid waste) and oily wastes
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a closed-system purge-and-trap process for analysis of VOCs in solid samples containing low levels (0.5 to 200 µg/kg) of VOCs. The method also provides specific procedures for preparation of samples containing high levels (>200 µg/kg) of VOCs. For low-level VOCs, a 5-g sample is collected into a vial that is placed into an autosampler device. Reagent water, surrogates and internal standards are added automatically, and the vial is heated to 40°C. The volatiles are purged into an appropriate trap using an inert gas combined with sample agitation. When purging is complete, the trap is heated and backflushed with helium to desorb the trapped sample components into a

GC for analysis. For high-level VOCs, samples are collected into a vial that contains a water-miscible organic solvent or a portion of sample is removed from the vial and dispersed in a water-miscible solvent. An aliquot of the solvent is added to reagent water, along with surrogates and internal standards, then purged and analyzed using an appropriate determinative method [e.g., SW-846 Method 8015D (Section 5.2.33) or 8260D (Section 5.2.34)].

Source: U.S. EPA. 2002. "Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples," Draft Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-5035a.pdf

5.2.27 EPA Method 6010D (SW-846): Inductively Coupled Plasma - Optical Emission Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-AES
Sample Preparation Method: EPA SW-846 Method 3015A (Section 5.2.16) for non-drinking water samples, EPA SW-846 Methods 3050B (Section 5.2.17) and 3051A (Section 5.2.18) for solid samples, and NIOSH Method 9102 (Section 5.2.80) for wipe samples
Sample Preparation Technique: Acid digestion

Method Developed for: Trace elements in solution
Method Selected for: This method has been selected for analysis of non-drinking water, solid and wipe samples to address the analytes listed in the table above as total arsenic, osmium, thallium, titanium or vanadium. It has also been selected for analysis of osmium tetroxide in drinking water. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits vary with each analyte and the specific instrument used. Instrument manufacturer documentation should be consulted for appropriate wavelengths, estimated instrument detection limits (IDLs) and analytical ranges. The upper end of the analytical range may be extended by sample dilution.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation

products, calcium and lead arsenate and sodium arsenite as total arsenic; osmium tetroxide as osmium; thallium sulfate as thallium; titanium tetrachloride as titanium; and ammonium metavanadate and vanadium pentoxide as total vanadium. Non-drinking water samples (prepared using SW-846 Method 3015A [Section 5.2.16]), soil samples (prepared using SW-846 Method 3050B [Section 5.2.17] or 3051A [Section 5.2.18]), and wipe samples (prepared using NIOSH Method 9102 [Section 5.2.80] or SW-846 Method 3051A [Section 5.2.18]) are analyzed by ICP-AES.

Special Considerations: This method uses hydrofluoric acid, which is highly toxic and penetrates the skin and tissues deeply if not treated immediately. Boric acid and/or other complexing reagents and appropriate treatment agents (e.g., benzalkonium chloride or calcium gluconate) should be administered immediately.[9] Concerns also have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit quantitation techniques when samples are analyzed using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, then lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 2014. "Method 6010D (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/6010d.pdf

5.2.28 EPA Method 6020B (SW-846): Inductively Coupled Plasma - Mass Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze a total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-MS
Sample Preparation Method: EPA SW-846 Method 3015A (Section 5.2.16) for non-drinking water samples, EPA SW-846 Methods 3050B (Section 5.2.17) and 3051A (Section 5.2.18) for solid samples, and NIOSH Method 9102 (Section 5.2.80) for wipe samples

[9] Medical management guidelines for hydrofluoric acid exposure are provided on the U.S. Centers for Disease Control and Prevention (CDC) National Institute for Occupational Safety and Health (NIOSH) Emergency Response Safety and Health Database at: https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750030.html

Sample Preparation Technique: Acid digestion

Method Developed for: Elements in water samples and in waste extracts or digests
Method Selected for: This method has been selected for analysis of non-drinking water, solid and wipe samples to address the analytes listed in the table above as total arsenic, osmium, thallium, titanium or vanadium. It has also been selected for analysis of osmium tetroxide in drinking water. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: IDLs, sensitivities and linear ranges vary with sample type, instrumentation and operation conditions. In relatively simple sample types, detection limits will generally be below 0.1 µg/L. Less sensitive elements, such as arsenic, may have detection limits of 1.0 µg/L or higher. The upper end of the analytical range may be extended by sample dilution.

Description of Method: This method will determine arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic. The method also will determine osmium tetroxide as total osmium, thallium sulfate as total thallium, titanium tetrachloride as titanium, and ammonium metavanadate and vanadium pentoxide as total vanadium. Water samples (prepared using SW-846 Method 3015A [Section 5.2.16]), soil samples (prepared using SW-846 Method 3050B [Section 5.2.17] or 3051A [Section 5.2.18), and wipe samples (prepared using NIOSH Method 9102 [Section 5.2.78] or SW-846 Method 3051A [Section 5.2.18]) are analyzed by ICP-MS.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, then lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: U.S. EPA. 2014. "Method 6020B (SW-846): Inductively Coupled Plasma-Mass Spectrometry," Revision 2. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/6020b.pdf

5.2.29 EPA Method 7470A (SW-846): Mercury in Liquid Wastes (Manual Cold-Vapor Technique)

Analyte(s)	CAS RN
This method has been selected to address the following analytes as total mercury if problems occur when using EPA Method 245.1 for preparation and analysis of non-drinking water samples.	
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Cold vapor atomic absorption spectrophotometry

Method Developed for: Mercury in mobility-procedure extracts, aqueous wastes and ground waters
Method Selected for: This method has been selected for use if problems occur when using EPA Method 245.1 for preparation and analysis of non-drinking water samples to address the analytes listed in the table above as total mercury. (See Footnote 12 of Appendix A.)
Detection and Quantitation: The detection limit for total mercury is 0.2 µg/L.

Description of Method: A 100-mL aqueous sample is digested with acids, permanganate solution, persulfate solution and heat. The sample is cooled and reduced with hydroxylamine-sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing the mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption spectrometry.

Special Considerations: Chloride and copper are potential interferences.

Source: U.S. EPA. 1994. "Method 7470A (SW-846): Mercury in Liquid Waste (Manual Cold-Vapor Technique)," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-7470a.pdf

5.2.30 EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique)

Analyte(s)	CAS RN
This method has been selected to address the following analytes as total mercury if problems occur when using EPA SW-846 Method 7473 for preparation and analysis of solid and wipe samples.	
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion for solid and non-drinking water samples, and acid digestion by NIOSH Method 9102 (Section 5.2.80) for wipe samples
Determinative Technique: Cold vapor atomic absorption spectrophotometry

Method Developed for: Total mercury in soils, sediments, bottom deposits and sludge-type materials
Method Selected for: This method has been selected for use if problems occur when using EPA SW-846 Method 7473 (Section 5.2.31) during preparation and analysis of solid and wipe samples to address the analytes listed in the table above as total mercury. (See Footnote 11 of Appendix A.)
Detection and Quantitation: Depending on the instrument used, the IDL for mercury is 0.2 µg/L.

Description of Method: A 0.5-g to 0.6-g sample is digested with aqua regia, permanganate solution and heat, then cooled and reduced with hydroxylamine-sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption spectrophotometry at a wavelength of 253.7 nm.

Special Considerations: Sulfides, chloride and copper are potential interferences.

Source: U.S. EPA. 2007. "Method 7471B (SW-846): Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)," Revision 2. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/7471b.pdf

5.2.31 EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry

Analyte(s)	CAS RN
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Thermal decomposition (solid samples) and acid digestion by NIOSH Method 9102 (wipe samples)
Determinative Technique: Visible spectrophotometry

Method Developed for: Total mercury in solids, aqueous samples and digested solutions
Method Selected for: This method has been selected for preparation of solid samples and for the analysis of prepared solid and wipe samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The IDL for total mercury is 0.01 ng. The typical working range is 0.05–600 ng, depending on the instrument used.

Description of Method: Controlled heating in an oxygenated decomposition furnace is used to liberate mercury from samples. The sample is dried and then thermally and chemically decomposed within the furnace. The decomposition products are carried by flowing oxygen to the catalytic section of the furnace, where oxidation is completed and halogens and nitrogen/sulfur oxides are trapped. The remaining decomposition products are then carried to an amalgamator that selectively traps mercury. After the system is flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator is rapidly heated, releasing mercury vapor. Flowing oxygen carries mercury vapor through absorbance cells positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance (peak height or peak area) is measured at 253.7 nanometers (nm) as a function of mercury concentration.

Special Considerations: If equipment is not available, use Method 7471B (EPA SW-846) for analysis of solid and wipe samples.

Source: U.S. EPA. 2007. "Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry," Revision 0. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-07/documents/epa-7473.pdf

5.2.32 EPA Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography

Analyte(s)	CAS RN
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid samples and water samples) and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: GC-NPD

Method Developed for: White phosphorus in soil, sediment and water
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address white phosphorus. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for reagent water, well water and pond water were calculated to be 0.008, 0.009 and 0.008 µg/L, respectively. MDLs for sand, sandy loam soil, and soil from the Rocky Mountain Arsenal were calculated to be 0.02, 0.43 and 0.07 µg/kg, respectively. This procedure provides sensitivity on the order of 0.01 µg/L for water samples and 1 µg/kg for soil samples.

Description of Method: Method 7580 can be used to determine the concentration of white phosphorus in soil, sediment and water samples using solvent extraction and GC. Water samples are extracted by one of two procedures, depending on the sensitivity required. For the more sensitive procedure, a 500-mL water sample is extracted with 50 mL of diethyl ether. The extract is concentrated by back extraction with reagent water, yielding a final extract volume of approximately 1.0 mL. A 1.0 µL aliquot of this extract is injected into a GC equipped with an NPD. Wet soil or sediment samples are analyzed by extracting a 40 g wet-weight aliquot of the sample with a mixture of 10.0 mL degassed reagent water and 10.0 mL isooctane. The extraction is performed in a glass jar on a platform shaker for 18 hours. A 1.0-µL aliquot

of the extract is analyzed by GC-NPD.

Special Considerations: The presence of white phosphorus should be confirmed using either a secondary GC column or an MS.

Source: U.S. EPA. 1996. "Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography," Revision 0. Washington, DC: U.S. EPA.
http://www.epa.gov/sites/production/files/2015-07/documents/epa-7580.pdf

5.2.33 EPA Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID

Analyte(s)	CAS RN
Diesel range organics	NA
Gasoline range organics	NA
Kerosene	64742-81-0

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-FID
Sample Preparation Method: EPA SW-846 Methods 3541 (Section 5.2.22)/3545A (Section 5.2.23) or 5035A (Section 5.2.26) for solid samples, 3520C (Section 5.2.20)/3535A (Section 5.2.21) or 5030C (Section 5.2.25) for water samples, and 3570 (Section 5.2.24)/8290A Appendix A (Section 5.2.36) for wipe samples. Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Sample Preparation Technique: Automated Soxhlet extraction / PFE / purge-and-trap (solid samples), SPE / purge-and-trap (water samples), and MSE / solvent extraction (wipe samples)

Method Developed for: Nonhalogenated VOCs and semivolatile organic compounds in water and soil samples
Method Selected for: This method has been selected for analysis of solid, water and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports that estimated MDLs vary with each analyte and range from 2 to 48 µg/L for aqueous samples. MDLs in other matrices have not been evaluated. The analytical range depends on the target analyte(s) and the instrument used.

Description of Method: This method provides GC conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds. Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques including purge-and-trap, direct injection of aqueous samples, and solvent extraction. An appropriate GC column and temperature program is used to separate the organic compounds, and the compounds are detected and measured by an FID.

Special Considerations: The presence of the analytes listed in the table above should be confirmed using either a secondary GC column or an MS.

Source: U.S. EPA. 2003. "Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID," Revision 4. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/8015d_r4.pdf

5.2.34 EPA Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography-Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Methyl acrylonitrile	126-98-7
Propylene oxide	75-56-9
The following analytes should be determined by this method (and corresponding sample preparation methods) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS
Sample Preparation Method: EPA SW-846 Methods 5035A (Section 5.2.26) for solid samples, 5030C (Section 5.2.25) for water samples, and 3570 (Section 5.2.24)/8290A Appendix A (Section 5.2.36) for wipes
Sample Preparation Technique: Purge-and-trap (solid samples and water samples) and MSE / solvent extraction (wipes)

Method Developed for: Applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, ground and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses (emulsified oil), tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils and sediments.
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. ***Note***: EPA Method 524.2 (Section 5.2.7), rather than 8260D (Section 5.2.34), should be used for analysis of acrylonitrile, carbon disulfide, 1,2-dichloroethane and methyl acrylonitrile in drinking water samples. EPA Method 524.2 also should be used for analysis of acrylonitrile and methyl acrylonitrile in non-drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports estimated quantitation limits (EQLs) of 5 µg/kg (wet weight) for soil/sediment samples and 5 µg/L for ground water, when using quadrupole instrumentation and purge-and-trap. The method also reports a lower limit of quantitation (LLOQ) of 0.02 µg/L for 1,2-dichloroethane. EQLs will be proportionately higher for sample extracts and samples that require dilution or when a reduced sample size is used to avoid saturation of the detector. The EQL for an individual analyte is dependent on the instrument as well as the choice of sample preparation/introduction method.

Description of Method: Volatile compounds are introduced into a GC by purge-and-trap or other procedures (see Section 1.2 of this method). The analytes can be introduced directly to a wide-bore capillary column or cryofocused on a capillary pre-column before being flash evaporated to a narrow-bore capillary for analysis. Alternatively, the effluent from the trap is sent to an injection port operating in the split mode for injection to a narrow-bore capillary column. The column is temperature-programmed to separate the analytes, which are then detected with an MS interfaced to the GC. Analytes eluted from the capillary column are introduced into the MS via a jet separator or a direct connection.

Source: U.S. EPA. 2006. "Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)." Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2017-04/documents/method_8260d_update_vi_final_03-13-

2017.pdf

5.2.35 EPA Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC-MS)

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Ethyldichloroarsine (ED)	598-14-1
Methyl paraoxon[1]	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phorate[1]	298-02-2
Phosphamidon	13171-21-6
Strychnine	57-24-9
Tetraethyl pyrophosphate (TEPP)	107-49-3
Trimethyl phosphite[2]	121-45-9

[1] If problems occur during measurement of oxon compounds, analysts should consider use of procedures included in Kamal, A. et al. "Oxidation of selected organophosphate pesticides during chlorination of simulated drinking water." *Water Research*. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/article/pii/S0043135408004995.

[2] If problems occur with analyses, lower the injection temperature.

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS
Sample Preparation Method: EPA SW-846 Methods 3541/3545A/3570 (solid samples), 3511/3520C/3535A (water samples), and 3570/8290A Appendix A or NIOSH Method 9102 (wipe samples). Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Sample Preparation Technique: Automated Soxhlet extraction/PFE/MSE (solid samples), continuous liquid-liquid extraction/SPE/MSE (water samples), and MSE/solvent extraction/acid digestion (wipe samples).

Method Developed for: Semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water samples
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Note:

- EPA Method 525.2 (Section 5.2.8) should be used to prepare and analyze drinking water samples for dichlorvos, disulfoton, fenamiphos and mevinphos; it also should be used to prepare and analyze non-drinking water samples for disulfoton.
- EPA/600/R-16/114 (Section 5.2.55) should be used to prepare and analyze solid and wipe samples for chlorfenvinphos, dichlorvos, dicrotophos, methyl paraoxon, methyl parathion, mevinphos, nicotine compounds, paraoxon, parathion, phorate, phosphamidon, strychnine and TEPP.

Detection and Quantitation: The method reports LLOQs in water ranging from 10 to 100 µg/L, depending on the analyte. EQLs reported in the method vary with each analyte and range between 10 and 40 µg/L for aqueous samples. Ranges are not provided for these analytes in soil samples. The analytical

range depends on the target analyte(s) and the instrument used.

Description of Method: Samples are prepared for analysis by GC-MS using the appropriate sample preparation and, if necessary, sample cleanup procedures. Semivolatile compounds are introduced into the GC-MS by injecting the sample extract into a GC with a narrow-bore fused-silica capillary column. The GC column is temperature-programmed to separate the analytes, which are then detected with an MS connected to the GC. Analytes eluted from the capillary column are introduced into the MS.

Special Considerations: Lower injection temperatures can alleviate problems that can occur with analysis of trimethyl phosphite.

Source: U.S. EPA. 2014. "Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)." Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2017-04/documents/method_8260d_update_vi_final_03-13-2017_0.pdf

5.2.36 EPA Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carbofuran (Furadan)	1563-66-2
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl hydrazine	60-34-4
Monocrotophos	6923-22-4
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Pentaerythritol tetranitrate (PETN)	78-11-5
Thiofanox	39196-18-4
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-NPD / GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Methods 7580 (Section 5.2.32), 8015D (Section 5.2.33), 8260D (Section 5.2.34), 8270E (Section 5.2.35), 8315A (Section 5.2.37), 8316 (Section 5.2.38), 8318A (Section 5.2.39), and 8330B (Section 5.2.40); EPA/600/R-15/097 (Section 5.2.53); ASTM Methods D7644-16 (Section 5.2.96) and D7645-16 (Section 5.2.97); adapted from Analyst, 126:1689-1693 (Section 5.2.107); adapted from J. Chromatogr. 617: 157-162 (Section 5.2.110); adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.116); adapted from J. Chromatogr. B, 874 (Section 5.2.117): 42-50; or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.119). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Evaluation of surface contamination by 2,3,7,8-substituted polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) congeners
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: A surface area of 2 inches by 1 foot is wiped with glass fiber paper saturated with distilled-in-glass acetone. One wipe is used per designated area. Wipes are combined into a single composite sample in an extraction jar and solvent extracted using a wrist action shaker.

Special Considerations: The solvent systems described in this extraction method have been evaluated for PCDD and PCDF congeners only. Other analytes may require different solvent systems for optimal sample extraction.

Source: U.S. EPA. 2007. "Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8290a.pdf

5.2.37 EPA Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid and non-drinking water samples) and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC

Method Developed for: Free carbonyl compounds in aqueous, soil, waste and stack samples
Method Selected for: This method has been selected for preparation and analysis of solid and non-drinking water samples to address formaldehyde. It has also been selected for analysis of prepared wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for formaldehyde varies depending on sample conditions and instrumentation, but is approximately 6.2 µg/L for reagent water.

Description of Method: A measured volume of aqueous sample (approximately 100 mL), or 100 mL of extract from an appropriate amount of solids (approximately 25 g), is buffered to pH 5 for analysis of formaldehyde, and derivatized with 2,4-dinitrophenylhydrazine (2,4-DNPH). Using the appropriate technique, the derivatives are extracted using methylene chloride and the extracts are exchanged with acetonitrile prior to HPLC analysis. HPLC conditions are described permitting the separation and measurement of various carbonyl compounds by absorbance detection at 360 nm.

Source: U.S. EPA. 1996. "Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8315a.pdf

5.2.38 EPA Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Acrylamide	79-06-1

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Direct injection (water samples), water extraction (solid), and MSE/solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-UV

Method Developed for: Acrylamide, acrylonitrile and acrolein in water samples
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid and wipe samples to address acrylamide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for acrylamide is 10 µg/L.

Description of Method: Samples are analyzed by HPLC. A 200-µL aliquot is injected onto a C_{18} reverse-phase column, and compounds in the effluent are detected with a UV detector. Water samples can be injected directly into the HPLC; solid samples must be extracted in water prior to injection.

Special Considerations: For details on method modifications allowing for the use of LC-MS-MS detection, please refer to the points of contact in Section 4.0.

Source: U.S. EPA. 1994. "Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8316.pdf

5.2.39 EPA Method 8318A (SW-846): *N*-Methylcarbamates by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid samples), and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-FL

Method Developed for: *N*-methylcarbamates in soil, water and waste matrices
Method Selected for: This method has been selected for preparation and/or analysis of solid and wipe

samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The estimated MDLs vary with each analyte and range from 1.7 to 9.4 µg/L for aqueous samples and 10 to 50 µg/kg for soil samples.

Description of Method: *N*-methylcarbamates are extracted from aqueous samples with methylene chloride, and from soils, oily solid waste and oils with acetonitrile. The extract solvent is exchanged to methanol/ethylene glycol, and the extract is cleaned using a C_{18} cartridge, filtered, and eluted on a C_{18} analytical column. After separation, the target analytes are hydrolyzed and derivatized post-column, then quantified fluorometrically. The sensitivity of the method usually depends on the level of interferences present, rather than on instrument conditions. Waste samples with a high level of extractable fluorescent compounds are expected to yield significantly higher detection limits.

Special Considerations: Techniques for analysis of these compounds in soil have been moving towards the use of LC-MS. Laboratories that are routinely using LC-MS for analysis of these compounds should consult with an appropriate contact in Section 4.0 regarding its use.

Source: U.S. EPA. 2007. "Method 8318A (SW-846): N-Methylcarbamates by High Performance Liquid Chromatography (HPLC)," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/8318a.pdf

5.2.40 EPA Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Pentaerythritol tetranitrate (PETN)	78-11-5
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction or direct injection (solid samples), SPE by EPA SW-846 Method 3535A (water samples), and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-UV

Method Developed for: Trace analysis of explosives and propellant residues in water, soil or sediment
Method Selected for: This method has been selected for preparation and/or analysis of solid, water and wipe samples to address the analytes listed in the table above. ***Note***: Methods 3535A (Section 5.2.21) and 8330B have been selected for preparation of water samples to address these analytes. For HMTD, procedures adapted from Analyst (2001) 126:1689-1693 (Section 5.2.107) have been selected for sample analysis. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits, ranges and interferences depend on the target compound.

Description of Method: This method is intended for the trace analysis of explosives and propellant residues by HPLC using a dual wavelength UV detector in a water, soil or sediment matrix. All of the compounds listed in this method either are used in the manufacture of explosives and propellants, or are the degradation products of compounds used for that purpose. Samples are prepared for analysis by

HPLC-UV detection using the appropriate sample preparation technique (SPE by Method 3535A or solvent extraction by Method 8330B) and, if necessary, sample cleanup procedures. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration. Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed.

Source: U.S. EPA. 2006. "Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)," Revision 2. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8330b.pdf

5.2.41 EPA ISM02.3 Cyanide: Analytical Methods for Total Cyanide Analysis

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Midi- or micro-distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Total cyanide in water, sediment, sludge and soil
Method Selected for: This method has been selected for preparation and analysis of solid, non-drinking water and wipe samples to address total cyanide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method quantitation limits for total cyanide are 10 µg/L for aqueous samples and 0.5 mg/kg for solid samples.

Description of Method: Cyanide is released as hydrocyanic acid from cyanide complexes by means of reflux-distillation, using either a midi- or micro-distillation process, and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined spectrophotometrically. In the semi-automated spectrophotometric measurement, cyanide is converted to cyanogen chloride without hydrolyzing to cyanate, by reaction with chloramine-T, at a pH less than 8. After the reaction is complete, color is formed on the addition of pyridine-barbituric acid reagent, and absorbance is read between 570 and 580 nm. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards.

Special Considerations: Midi-distillation is recommended for soil samples to mitigate low analyte recoveries that can occur when analyzing these sample types. If the appropriate equipment is available, the in-line distillation procedure described in EPA-821-B-01-009 (Section 5.2.59) can be used when preparing and analyzing aqueous samples, to shorten analysis time and reduce matrix interferences.

Source: U.S. EPA. "ISM02.3: Exhibit D – Part D: Analytical Methods for Total Cyanide Analysis." Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-10/documents/ism23d.pdf

5.2.42 EPA Method 3135.2l: Cyanide, Total and Amenable in Aqueous and Solid Samples Automated Colorimetric With Manual Digestion

Analyte(s)	CAS RN
Cyanide, Amenable to chlorination	NA

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion followed by distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Cyanide in drinking, ground and surface waters, domestic and industrial wastewaters, sediments and solid waste
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address cyanide amenable to chlorination. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The applicable range is 0.003–0.500 mg/L cyanide in the distillate. This range can be expanded either by using less sample for distillation or by diluting the distillate.

Description of Method: This method detects inorganic cyanides that are present as either simple soluble salts or complex radicals. It may be used to determine values for both total cyanide and cyanide amenable to chlorination (also known as available cyanide). Cyanide is released as hydrocyanic acid by refluxing a sample with strong acid. The hydrocyanic acid is distilled and collected in an absorber-scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by automated colorimetry. For determination of cyanide amenable to chlorination, a portion of the sample is chlorinated using sodium hypochlorite at a pH > 11 to decompose the cyanide. Cyanide levels are then determined in both the chlorinated sample portion of the sample and a portion of the sample that has not been chlorinated using the total cyanide method. Cyanides amenable to chlorination are then calculated by difference between unchlorinated and the chlorinated aliquots of the sample.

Special Considerations: Alternate cyanide analyzer equipment may be used, provided it is used according to the procedures described and the laboratory can demonstrate equivalent performance. If preferred, Standard Method 4500-CN-G (Section 5.2.102) can be used in place of this method for the analysis of cyanide amenable to chlorination in water samples.

Source: Greenlee, A. 2008. "RLAB Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Soil Samples Automated Colorimetric With Manual Digestion." Lenexa, KS: U.S. EPA Region 7 Laboratory. http://www.epa.gov/sites/production/files/2015-07/documents/epa-3135.2i.pdf

5.2.43 EPA IO [Inorganic] Compendium Method IO-3.1: Selection, Preparation, and Extraction of Filter Material

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid extraction
Determinative Technique: ICP-AES / ICP-MS

Determinative Method: EPA Method IO-3.4 (osmium tetroxide) or Methods IO3.4/IO-3.5 (all other analytes)

Method Developed for: Particulate metals in air
Method Selected for: This method has been selected for preparation of air samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method supports determination of arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate, and sodium arsenite as total arsenic. Thallium sulfate is determined as total thallium, ammonium metavanadate and vanadium pentoxide are determined as total vanadium, and osmium tetroxide is determined as total osmium. A subsample (one-ninth of the overall filter) is obtained by cutting a strip from the filter used to collect the sample. The filter strip is extracted using a hydrochloric/nitric acid mix and microwave or hotplate heating. The extract is filtered, worked up to 20 mL, and analyzed using either Method IO-3.4 (Section 5.2.44) or Method IO-3.5 (Section 5.2.45).

Source: Mainey, A. and Winberry, W.T. 1999. "IO Compendium Method IO-3.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Selection, Preparation and Extraction of Filter Material." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a.
http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.1.pdf

5.2.44 EPA IO [Inorganic] Compendium Method IO-3.4: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-AES
Sample Preparation Method: EPA Method IO-3.1
Sample Preparation Technique: Acid extraction

Method Developed for: Metals in ambient particulate matter
Method Selected for: This method has been selected for analysis of prepared air samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate, and sodium arsenite as total arsenic. Osmium tetroxide is determined as total osmium, thallium sulfate is determined as total thallium, and ammonium metavanadate and vanadium pentoxide are determined as total vanadium. Ambient air is sampled by high-volume filters using Method IO-2.1 and the filters are extracted by Method IO-3.1 (Section 5.2.43). Detection limits, ranges and interference corrections are dependent on the analyte and the instrument used.

Special Considerations: Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative.

Sources: Winberry, W.T. 1999. "IO Compendium Method IO-3.4: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.4.pdf

Winberry, W.T. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

5.2.45 EPA IO [Inorganic] Compendium Method IO-3.5: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP-MS)

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-MS
Sample Preparation Method: EPA Method IO-3.1
Sample Preparation Technique: Acid extraction

Method Developed for: Metals in ambient particulate matter
Method Selected for: This method has been selected for analysis of prepared air samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits, ranges and interference corrections are dependent on the

analyte and the instrument used.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate, and sodium arsenite as total arsenic. Thallium sulfate is determined as total thallium, and ammonium metavanadate and vanadium pentoxide are determined as total vanadium. Ambient air is sampled by high-volume filters using Method IO-2.1 (a sampling method). The filters are extracted by Method IO-3.1 (see Section 5.2.43).

Source: Winberry, W.T. 1999. "IO Compendium Method IO-3.5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.5.pdf

Winberry, W.T. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

5.2.46 EPA IO [Inorganic] Compendium Method IO-5: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)

Analyte(s)	CAS RN
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion for particulate mercury
Determinative Technique: Cold vapor atomic fluorescence spectrometry (CVAFS)

Method Developed for: Vapor and particle phase mercury in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits are 30 pg/m^3 for particulate mercury and 45 pg/m^3 for vapor phase mercury. Detection limits, analytical range and interferences are dependent on the instrument used.

Description of Method: Vapor phase mercury is collected using gold-coated glass bead traps at a flow rate of 0.3 L/minute. The traps are directly desorbed onto a second (analytical) trap, and the desorbed mercury is determined by CVAFS. Particulate mercury is sampled on glass-fiber filters at a flow rate of 30 L/minute. The filters are extracted with nitric acid and microwave heating, and the extract is oxidized with bromine chloride, then reduced with stannous chloride and purged from solution onto a gold-coated glass bead trap. This trap is desorbed onto a second trap, the second trap is desorbed, and the mercury is determined by CVAFS.

Special Considerations: There are no known positive interferences at 253.7 nm wavelength. Water vapor will cause a negative interference.

Source: Keele, G. and Barres, J. 1999. "IO Compendium Method IO-5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-5.pdf

5.2.47 EPA Air Method, Toxic Organics - 10A (TO-10 A): Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)

Analyte(s)	CAS RN
BZ [Quinuclidinyl benzilate][1]	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol[2]	96-24-2
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)[2]	1445-75-6
Dimethylphosphite	868-85-9
Dimethylphosphoramidic acid[1]	33876-51-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid][1]	73207-98-4
Ethyl methylphosphonic acid (EMPA)[1]	1832-53-7
Fenamiphos	22224-92-6
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Methylphosphonic acid (MPA)[1]	993-13-5
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8
Phosphamidon	13171-21-6
Pinacolyl methyl phosphonic acid (PMPA)[1]	616-52-4
Tetraethyl pyrophosphate (TEPP)	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Trimethyl phosphite	121-45-9
The following analyte should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-15.	
Allyl alcohol	107-18-6

[1] For this analyte, HPLC is the preferred technique; however, if problems occur, Method TO-10A must be modified to include a derivatization step prior to analysis by GC-MS.
[2] If problems occur when using this method, it is recommended that Method TO-15 be used.

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS or HPLC-UV

Method Developed for: Pesticides and polychlorinated biphenyls in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The limit of detection (LOD) will depend on the specific compound measured, the concentration level, and the degree of specificity required. This method is applicable to multicomponent atmospheres, 0.001–50 µg/m^3 concentrations, and 4–24-hour sampling periods.

Description of Method: A low-volume sample collection rate (1–5 L/minute) is used to collect vapors on a sorbent cartridge containing polyurethane foam (PUF) in combination with another solid sorbent. Airborne particles also are collected, but the sampling efficiency for particulates is not known. Pesticides and other chemicals are extracted from the sorbent cartridge with 5% diethyl ether in hexane, and determined by GC-MS. For common pesticides, HPLC coupled with a UV detector is preferable. HPLC-UV is also the preferred technique for BZ, dimethylphosphoramidic acid, EA2192, EMPA, IMPA, MPA and PMPA.

Special Considerations: Refer to footnotes provided in the analyte table above for special considerations that should be applied when measuring specific analytes.

Source: Lewis, R.G. 1999. "Air Method, Toxic Organics-10A (TO-10A): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air: Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)." Cincinnati, OH: U.S. EPA. EPA 625/R-96/010b. https://www.epa.gov/sites/default/files/2015-07/documents/epa-to-10a.pdf

Additional Resource: Karmel, A., Byrne, C., Bigo, C., Ferrario, J., Stafford, C., Verdin, G., Siegelman, F., Knizner, S. and Hetrick, J.. 2009. "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 43(2): 522-534. http://www.sciencedirect.com/science/article/pii/S0043135408004995

5.2.48 EPA Air Method, Toxic Organics - 15 (TO-15): Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethyldichloroarsine (ED)	598-14-1
Ethylene oxide	75-21-8
The following analytes should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-10A or TO-17.	
3-Chloro-1,2-propanediol	96-24-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Diisopropyl methylphosphonate (DIMP)	1445-75-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Sarin (GB)	107-44-8
Soman (GD)	96-64-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected using canisters
Determinative Technique: GC-MS

Method Developed for: VOCs in air

Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method applies to ambient concentrations of VOCs above 0.5 parts per billion by volume (ppbv) and typically requires VOC enrichment by concentrating up to 1 L of a sample volume; however, when using current technologies, quantifications of approximately 100 parts per trillion by volume (pptv) have been achieved with 0.5-L sample volumes.

Description of Method: The atmosphere is sampled by introduction of air into a specially prepared stainless steel canister (electropolished or silica-coated). A sample of air is drawn through a sampling train comprising components that regulate the rate and duration of sampling into the pre-evacuated and passivated canister. Grab samples also may be collected. After the air sample is collected, the canister valve is closed, an identification tag is attached to the canister, and the canister is transported to the laboratory for analysis. To analyze the sample, a known sample volume is directed from the canister through a solid multisorbent concentrator. Recovery of less volatile compounds may require heating the canister. After the concentration and drying steps are completed, VOCs are thermally desorbed, entrained in a carrier gas stream, and then focused in a small volume by trapping on a cryo-focusing (ultra-low temperature) trap or small volume multisorbent trap. The sample is then released by thermal desorption and analyzed by GC-MS.

Special Considerations: If problems occur when using this method for determination of allyl alcohol, it is recommended that Method TO-10A (Section 5.2.47) be used. In cases where lower detection levels are needed, use procedures included in the supplement to EPA Compendium Method TO-15: Reduction of Method Detection Limits to Meet Vapor Intrusion Monitoring Needs (https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100R6QV.txt).

Source: McClenny, W.A. and Holdren, M.W. 1999. "Air Method, Toxic Organics-15 (TO-15): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Second Edition: Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)." Cincinnati, OH: U.S. EPA. EPA 625/R-96/010b.
http://wipp.energy.gov/library/Information_Repository_A/Supplemental_Information/EPA%201999/TO-15.pdf

5.2.49 EPA Air Method, Toxic Organics – 17 (TO-17): Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes

Analyte(s)	CAS RN
A-230 (Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate)	2387496-12-8
A-232 (Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-04-8
A-234 (Ethyl N-[(1E)-1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-06-0
Chlorosarin*	1445-76-7
Chlorosoman*	7040-57-5
Cyclohexyl sarin (GF)	329-99-7
1-Methylethyl ester ethylphosphonofluoridic acid (GE)*	1189-87-3
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard sulfur / Mustard gas (HD)	505-60-2
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Sarin (GB)*	107-44-8
Soman (GD)*	96-64-0
Tabun (GA)	77-81-6
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

*If problems occur when using this method, it is recommended that Method TO-15 be used.

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Thermal desorption
Determinative Technique: GC-MS

Method Developed for: VOCs
Method Selected for: This method has been selected for preparation and analysis of CWAs in air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD will depend on the specific compounds measured, the concentration level, and the degree of specificity required. This method is applicable to multicomponent atmospheres, 2.86 to 275 µg/m^3 concentrations, and 1 to 24-hour sampling periods.

Description of Method: A low-volume (10 to 200 mL/minute) sample collection rate is used to collect vapors on a sorbent tube. Airborne particles also are collected, but the sampling efficiency for particulates is not known. Compounds are then thermally desorbed from the sorbent tube and determined by GC-MS.

Special Considerations: Refer to the footnote provided in the analyte table above for special considerations that should be applied when measuring specific analytes. Higher volume sampling flow rates can be used for high boiling materials such as the V-agents.

Source: Woolfenden, E.A. and McClenny, W.A. 1999. "Air Method, Toxic Organics-17 (TO-17): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air: Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010b.
https://www3.epa.gov/ttnamti1/files/ambient/airtox/to-17r.pdf

5.2.50 EPA/600/R-11/143: Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Extracted using sonication, and filtered using a syringe-polyvinylidene fluoride (PVDF) filter unit
Determinative Technique: LC-MS-MS

Method Developed for: TEA, EDEA and MDEA in wipe surfaces
Method Selected for: This method has been selected for preparation and analysis of wipe samples and for the analysis of prepared solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers. ***Note***: SW-846 Methods 3541/3545A should be used for preparation of solid samples.
Detection and Quantitation: Detection limits (DL) for EDEA, MDEA and TEA are 0.06, 0.07 and 0.12 ng/cm^2, respectively. The limits of quantitation (LOQs) for EDEA, MDEA and TEA are 0.63, 0.69, and 1.23 ng/cm^2, respectively. The reporting range for all three target compounds is 0.1–5.0 ng/cm^2.

Description of Method: Samples are collected from surfaces with wipes and stored at 0–6°C if not analyzed within 24 hours. Samples are brought to ambient temperature, then spiked with a surrogate compound and solvent. Samples are then sonicated, extracted with a syringe filter unit, concentrated, and analyzed directly by LC-MS-MS in the positive electrospray ionization (ESI+) mode. Each target compound is separated and identified by retention time and by comparing the sample primary multiple reaction monitoring (MRM) transition to the known standard MRM transition from reference spectra under identical LC-MS-MS conditions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. The concentration of each analyte is determined by the instrumentation software using external calibration.

Special Considerations: A more recent procedure based on this method is provided in the additional resource cited below. This procedure uses a lower calibration curve and modified LC-MS-MS instrument conditions.

Source: U.S. EPA and CDC. 2011. "Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-11/143.
https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc%2F&dirEntryId=238641

Additional Resource: Dynamac Corporation. 2012. "Standard Operating Procedure for the Determination of Ethanolamines," Dynamac SOP L-A-303 Rev. 2. Copies of this analytical protocol may be requested at https://www.epa.gov/homeland-security-research/forms/contact-us-about-homeland-security-research.

5.2.51 EPA/600/R-12/653: Verification of Methods for Selected Chemical Warfare Agents (CWAs)

Analyte(s)	CAS RN
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Tabun (GA)	77-81-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: HN-1, HN-3, VR and GA in solid, water and/or wipe samples
Method Selected for: This method has been selected for preparation and analysis of solid, water and/or wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: IDLs for GA, HN-1, HN-3 and R-33, respectively, are: 0.8, 0.025, 0.025 and 0.025 ng/µL (GC-MS full scan); 0.1, 0.01, 0.01 and 0.01 ng/µL (GC-MS SIM); and 0.1, 0.025, 0.005 and 0.02 ng/µL (GC-MS time of flight [TOF]). IDLs for GC-MS full-scan using the total ion chromatogram (TIC) for GA, HN-1, HN-3, and R 33, respectively, are: 0.4, 0.05, 0.025 and 0.025 ng/µL.

- MDLs for GC-MS full-scan in soils ranged from 8 to 106 µg/kg for GA, 35 to 81 µg/kg for HN-1, 57 to 243 µg/kg for HN-3, and 81 to 213 µg/kg for R 33.
- MDLs for GC-MS TOF in soils ranged from 0.33 to 0.39 µg/kg for GA, 0.57 to 2.3 µg/kg for HN-1, 1.6 to 12 µg/kg for HN-3, and 15 to 49 µg/kg for R 33.
- MDLs for GA, HN-1, HN-3 and R 33 in reagent water using GC-MS full-scan are 16, 1.8, 20 and 69 µg/L, respectively.
- MDLs for GA, HN-1, HN-3 and R 33 in reagent water using GC-MS TOF are 0.13, 0.084, 0.72 and 22 µg/L, respectively.
- MDLs for GA, HN-1, HN-3 and R 33 in wipes using GC-MS full-scan are 0.11, 0.023, 0.35 and 4.41 ng/cm^2, respectively.

Calibration ranges for GC-MS full scan are 0.025–1.0 ng/µL for GA, HN-1 and HN-3 and 0.8–3.0 ng/µL for R 33. Calibration ranges for GC-MS SIM are 0.01–0.25 ng/µL for GA, HN-1 and HN-3 and 0.1–1.0 ng/µL for R 33. Calibration ranges for GC-MS TOF are 0.01–1 ng/µL for GA, HN-1 and HN-3 and 0.10–5 ng/µL for R 33.

Description of Method: Water samples are extracted by adding ~8.8 g of sodium chloride to 35-mL of water sample. Surrogates and 2 mL of methylene chloride are added. The samples are extracted on a shaker table for 2 minutes and the layers are allowed to separate. The methylene chloride layer is collected, dried with anhydrous sodium sulfate, and 1 mL is transferred to an autosampler vial. Solid samples are extracted by mixing 10 g of solid, 2.5 g of anhydrous sodium sulfate, 5–10 glass beads, and 25 mL of a 25/50/25 (v/v/v) mixture of acetone/methylene chloride/ethyl acetate. The solid samples are then sonicated in a water bath for 1 hour. The extract is retained, and dried with an additional 1–2 g of sodium sulfate. The sample is then re-extracted by water bath sonication for an additional hour using 25 mL of 5% TEA/95% ethyl acetate. The second extract is retained and dried with 1–2 g of sodium sulfate. The first and second extracts are separately reduced in volume under nitrogen, and 1 mL of each extract is transferred to a separate autosampler vial. Internal standards are added to both extracts, and the extracts are analyzed by GC-MS.

Special Considerations: During method development studies for the analytical protocol described in the EPA/600/R-16/114 (Section 5.2.55), ethyl acetate and TEA were found to produce chromatographic interferences. In addition, ethyl acetate has a higher boiling point than other solvents (e.g., methylene chloride), resulting in a longer nitrogen blowdown step than if other solvents are used. Alternative solvent systems used for similar compounds (see methods in Sections 5.2.56 and 5.2.57) may result in improved chromatography. The method has been single-laboratory tested in reagent water, sand, soil and wipes. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2013. "Verification of Methods for Selected Chemical Warfare Agents (CWAs)." Washington, DC: U.S. EPA. EPA/600/R-12/653.
https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=248575

5.2.52 EPA/600/R-13/224: Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP) (degradation product of GB)	1445-75-6
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-54-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using sonication and filtered using a syringe-PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IMPA, MPA and PMPA in wipe samples
Method Selected for: This method has been selected for analysis of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The working range of this method is 0.10–3.0 ng/cm^2 for EMPA, 0.25–7.50 ng/cm^2 for IMPA and MPA, and 0.05–1.5 ng/cm^2 for DIMP and MPA. MDLs obtained from wiping a laminate surface for EMPA, IMPA, MPA and PMPA are reported as 0.05, 0.04, 0.07 and 0.02 ng/cm^2, respectively. Method reporting limits for EMPA, IMPA, MPA and PMPA are 0.1, 0.25, 0.25 and 0.05 ng/cm^2, respectively.

Description of Method: Wipe samples are spiked with surrogates and 5-mL of LC-MS grade water. The sample solution is then sonicated and extracted with a syringe filter unit, and the extract is analyzed directly by LC-MS-MS operated simultaneously in positive and negative ESI modes. Each target compound is separated chromatographically and identified by retention time and by comparison of the primary MRM transition for the sample to the reference spectra of MRM transition for known standards. The concentration of each analyte is determined using external calibration. Surrogates are used to monitor extraction efficiency.

Special Considerations: This procedure uses cotton gauze wipes, which were determined to provide the highest analyte recoveries with the least interference. Other wipes, such as filter paper or glass fiber filters had comparable recoveries and could be appropriate alternatives, but are not as sturdy. Data described in this procedure refer to ESI- mode because some complications can occur in ESI+ mode. Recoveries of DIMP may be problematic due to the volatility or rapid decomposition. Because wood surfaces resulted in

poor recoveries (likely due to surface porosity), the method recommends that it should not be used to identify these analytes on wood surfaces. The method does not include analysis of dimethylphosphoramidic acid, and method modifications (e.g., pH adjustment) may be needed when analyzing samples for this compound.

Source: U.S. EPA and CDC. 2013. "Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-13/224. https://www.hsdl.org/?abstract&did=746488

5.2.53 EPA/600/R-15/097: Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Direct injection (water samples), EPA SW-846 Method 3541/3545A (solid samples) and EPA SW-846 Method 3570/8290A Appendix A (wipes)
Determinative Technique: LC-MS-MS

Method Developed for: EA2192 in water
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid samples and wipes to address EA2192. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The detection limit for EA2192 in deionized water is 0.0130 µg/L. The minimum reporting level in deionized water is 0.125 µg/L. The suggested calibration range is 0.05–20 µg/L.

Description of Method: A 40-mL water sample is collected in a bottle containing sodium omadine (antimicrobial agent) and ammonium acetate. An aliquot of sample is placed in an autosampler vial with the internal standard added. A 50-µL injection is made into an LC equipped with a C_{18} column interfaced to an MS-MS operated in ESI+ mode. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by internal standard calibration using procedural standards.

Special Considerations: The method has been tested in deionized water and various drinking waters, including chlorinated and chloraminated surface and ground waters. EA2192 is highly toxic; therefore, the procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-15/097. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=311259

5.2.54 EPA/600/R-15/258: Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA)*	85090-33-1
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]*	40334-70-1
Lewisite oxide*	1306-02-1

* In cases where standards are not available or increased sample throughput is needed, these compounds also can be addressed by analyzing samples for total arsenic (see Appendix A for appropriate ICP-AES or -MS methods).

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: LC-MS-MS

Method Developed for: Extraction and analysis of lewisite 1 by its degradation products (CVAA and CVAOA)
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method detects both CVAA and CVAOA as total CVAOA. Detection limits for CVAOA are 0.041 mg/L for water, 0.38 µg/wipe for wipes, 0.073 µg/g for sand, 0.032 µg/g for Nebraska soil, 0.028 µg/g for Virginia soil and 0.055 µg/g for Georgia soil. The suggested calibration range for CVAOA is 0.02–0.2 µg/mL.

Description of Method: Phenyl arsonous acid is added to all samples as a surrogate prior to sample extraction and analysis. Water samples are mixed thoroughly. An acidified (with hydrochloric acid) methanolic solution is added to soil samples, followed by agitation on a shaker table for 30 minutes. Soil samples are then allowed to settle by gravity before an aliquot of the extract (liquid layer) is taken. A dilute aqueous solution of hydrochloric acid is added to wipe samples followed by agitation on a shaker table for 30 minutes. Prior to analysis by LC-MS-MS, hydrogen peroxide is added to all extracts to completely degrade target analytes to CVAOA and the surrogate to phenyl arsenic acid.

Special Considerations: In cases where standards are not available or increased sample throughput is needed, these compounds also can be addressed by analyzing samples for total arsenic (see Appendix A for appropriate ICP-AES or -MS methods and corresponding method usability tiers).

Source: U.S. EPA. 2015. "Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)," Revision 1. Washington, DC: U.S. EPA. EPA/600/R-15/258. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=310272

5.2.55 EPA/600/R-16/114: Analytical Protocol for Measurement of Extractable Semivolatile Organic Compounds Using Gas Chromatography/Mass Spectrometry

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
Chloropicrin[1]	76-06-2
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Crimidine	535-89-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethylphosphite	868-85-9
Disulfoton	298-04-4
Disulfoton sulfone oxon	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon	2496-92-6
1,4-Dithiane	505-29-3
Fenamiphos	22224-92-6
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8
Phosphamidon	13171-21-6
Strychnine	57-24-9
Tetraethyl pyrophosphate (TEPP)	107-49-3
Tetramethylenedisulfotetramine	80-12-6
1,4-Thioxane[2]	15980-15-1

[1] If problems occur with analyses, lower the injection temperature.
[2] If problems occur when using this method, it is recommended that SW-846 Method 8260D [Section 5.2.34] and appropriate corresponding sample preparation procedures (i.e., Method 5035A [Section 5.2.26] for solid samples and Method 5030C [Section 5.2.25] for water samples) be used.

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Semivolatile organic compounds in extracts prepared from solid waste matrices, soils, air sampling media and water samples
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Note:

- EPA Method 525.2 (Section 5.2.8) has been selected for preparation and analysis of drinking water samples for chlorpyrifos, dichlorvos, fenamiphos and mevinphos.
- EPA Method 525.2 (Section 5.2.8) also has been selected for preparation and analysis of water samples for disulfoton, disulfoton sulfone oxon, disulfoton sulfoxide and disulfoton sulfoxide oxon.

- EPA Method 525.3 (Section 5.2.9) has been selected for preparation and analysis of drinking water samples for phosphamidon.
- EPA Method 540 (Section 5.2.12) has been selected for preparation and analysis of water samples for chlorpyrifos oxon, phorate sulfone, phorate sulfone oxon, phorate sulfoxide and phorate sulfoxide oxon.
- EPA Method 551.1 (Section 5.2.14) has been selected for preparation and analysis of water samples for chloropicrin.
- SW-846 Method 8270E (Section 5.2.35) has been selected for analysis of prepared water samples for chlorfenvinphos, dicrotophos, methyl paraoxon, methyl parathion, nicotine compounds, paraoxon, parathion, phorate, strychnine and TEPP (see Appendix A for the appropriate sample preparation methods).
- SW-846 Method 8270E (Section 5.2.35) also has been selected for analysis of prepared non-drinking water samples for dichlorvos, mevinphos and phosphamidon (see Appendix A for the appropriate sample preparation methods).

All other analyte/sample type combinations should be prepared and analyzed by this method.

Detection and Quantitation: MDL and Quantitation Limit (QL) ranges, when performing full-scan analysis of aqueous samples, are 0.79–4.0 and 28.6–286 µg/L, respectively; and 1.6–93.1 µg/L and 50–1200 µg/kg, respectively, for soil samples. MDL and QL ranges when performing SIM analysis of aqueous samples are 0.030–1.45 and 0.23–114 µg/L, respectively; and 0.047–15.4 and 0.4–80 µg/kg, respectively, for soil samples. The analytical range depends on the target analyte(s) and the mode of analysis used (i.e., full-scan or SIM).

Description of Method: Prior to analysis, surrogates, sodium chloride and methylene chloride are added to aqueous, soil and wipe samples and prepared by MSE. Extracts are dried by the addition of sodium sulfate, concentrated (if necessary to achieve appropriate detection and quantitation) by nitrogen evaporation, and then analyzed by GC-MS in full-scan or SIM mode.

Special Considerations: Laboratory results indicate that improved recovery of alkaline compounds (e.g., strychnine, nicotine compounds, crimidine, and phencyclidine) from water may result when extracting samples under acidic conditions (e.g., pH <2) during the first extraction, followed by back extraction under basic conditions. If problems occur with the analysis of chloropicrin, lower the injection temperature. If problems occur when analyzing for 1,4-thioxane, it is recommended that SW-846 Method 8260D [Section 5.2.34] and appropriate corresponding sample preparation procedures (i.e., Method 5035A [Section 5.2.26] for solid samples or Method 5030C [Section 5.2.25] for water samples) be used.

Source: U.S. EPA. 2016. "Analytical Protocol for Measurement of Extractable Semivolatile Organic Compounds Using Gas Chromatography/Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-16/114. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=532353

5.2.56 EPA/600/R-16/115: Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry

Analyte(s)	CAS RN
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl sarin (GF)	329-99-7
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mustard, sulfur / Mustard gas (HD)	505-60-2
Sarin (GB)	107-44-8
Soman (GD)	96-64-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Determination of GF, GB, GD and HD in water, soil and wipes
Method Selected for: This method has been selected for preparation and analysis of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The calibration ranges in full scan mode are 11.4–114 µg/L (GB and GF) and 5.7–57 µg/L (GD and HD) for water samples, 20–200 µg/kg (GB and GF) and 10–100 µg/kg (GD and HD) for soil samples, and 0.02–0.2 µg/cm^2 (GB and GF) and 0.01–0.1 µg/cm^2 (GD and HD) for wipes.

Description of Method: The method involves solvent extraction of the sample followed by GC-MS analysis to determine cyclohexyl sarin, sarin, soman and HD in water, soil and wipes. Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each sample type. Aqueous, solid and wipe samples are spiked with surrogates and extracted by microscale extraction, using methylene chloride. Water is removed from extracts with anhydrous sodium sulfate, and the extracts are concentrated (solids and wipe extracts only) by nitrogen evaporation, then analyzed by GC-MS using a mass selective detector in either full scan mode or TOF.

Special Considerations: This method has been tested in multiple laboratories for analysis of cyclohexyl sarin, sarin, soman and HD in reagent water, drinking water, ground water, sand and wipes. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-16/115. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=532354

5.2.57 EPA/600/R-16/116: Analytical Protocol for VX Using Gas Chromatography/Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Determination of VX in water, soil and wipes
Method Selected for: This method has been selected for preparation and analysis of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The calibration ranges for analysis of VX using full scan mode are 11.4–114 µg/L for water samples, 20–200 µg/kg for soil samples, and 0.02–0.2 µg/cm^2 for wipes.

Description of Method: The method involves micro-scale solvent extraction of samples followed by

GC-MS analysis. Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each sample type. Aqueous and wipe samples are spiked with surrogates and extracted using methylene chloride. Solid samples are spiked with surrogates, then extracted first using a Tris buffer solution, followed by extraction with methylene chloride. Water is removed from extracts using anhydrous sodium sulfate, and the extracts are concentrated (solids and wipe extracts only) by nitrogen evaporation, then analyzed by GC-MS using either a mass selective detector in full scan mode or TOF.

Special Considerations: The method has been tested in multiple laboratories for analysis of VX in reagent water, drinking water, ground water, soil and wipes. Laboratory data indicate some difficulties with analyte recoveries in soil; modifications might be needed for application of the procedures to various soil types. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Analytical Protocol for VX Using Gas Chromatography/Mass Spectrometry (GC/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-16/116.
https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=337633

5.2.58 EPA/600/R-18/056: Direct Aqueous Injection of the Fluoroacetate Anion in Potable Water for Analysis by Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection
Determinative Technique: LC-MS-MS

Method Developed for: Analytes containing fluoroacetate anion (FAA) in water
Method Selected for: This procedure has been selected for preparation and analysis of water samples to address fluoroacetic acid, fluoroacetate salts and methyl fluoroacetate. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoroacetate anion in reagent grade water is 0.4 µg/L. The minimum reporting level in reagent grade water is 0.65 µg/L. The suggested calibration range is 1 – 100 µg/L.

Description of Method: This report describes a procedure for analysis of analytes containing fluoroacetate anion in water samples, and the results of testing the procedure for analysis of fluoroacetic acid and methyl fluoroacetate as the fluoroacetate anion in drinking water. A 40-mL water sample is collected in a bottle containing ascorbic acid (chlorine neutralizer) and sodium omadine (anti-microbial agent). pH adjustment may be needed if methyl fluoroacetate is the target analyte (see Special Considerations). An aliquot of sample (990 µL) is filtered through a 0.22-µm filter into an autosampler vial containing 10 µL of an internal standard solution. A 20-µL injection is made into an LC equipped with a C_8 column interfaced to an MS-MS operated in ESI- mode. Analytes are separated and identified as the fluoroacetate anion by comparing the acquired mass spectra and retention times to reference spectra and retention times acquired under identical LC-MS-MS conditions for calibration standards. The concentration of the anion is determined by isotope dilution.

Special Considerations: Methyl fluoroacetate (MFA) is subject to both acid- and base-hydrolysis in water, forming the free acid, FAA. Preliminary experiments were conducted to verify hydrolysis of MFA to FFA in water, examine the effect of water pH on the hydrolysis, and determine whether FAA measurements would be a feasible way to characterize MFA contamination levels. The method was then

tested for analysis of MFA in deionized water and four different drinking waters, with and without preservative. In unpreserved water, MFA is completely converted to FAA over the course of 24 hours. In preserved water, the pH was considered too low (<6.5) to facilitate hydrolysis. To ensure complete hydrolysis of MFA to FAA, the pH of the water sample should be adjusted to greater than 8 and shaken vigorously for longer than 24 hours.

Source: EPA. 2018. "Direct Aqueous Injection of the Fluoroacetate Anion in Potable Water in Potable Water for Analysis by Liquid Chromatography/Tandem Mass Spectrometry." EPA/600/R-18/056. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=343292&Lab=NHSRC&subject=Homeland%20Security%20Research&view=desc&sortby=pubDateYear&showcriteria=1&count=25

Additional Resource: Parry, E. and Willison, S. 2018. "Direct Aqueous Injection of the Fluoroacetate Anion in Potable Water in Potable Water for Analysis by Liquid Chromatography/Tandem Mass Spectrometry." *Analytical Methods*. 10(46): 5455-5590. RSC Publishing, Cambridge, United Kingdom https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6309164/

5.2.59 EPA-821-B-01-009: Method Kelada-01: Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: On-line UV irradiation followed by flash distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Total cyanide, acid dissociable cyanide and thiocyanate in water, sediment, sludge and soil
Method Selected for: This method has been selected as an alternative to ISM02.3 for preparation and analysis of non-drinking water samples to address total cyanide.
Detection and Quantitation: The LOD is 0.5 µg/L. The working range is 0–100 µg/L.

Description of Method: The method uses a combined on-line UV irradiation and flash distillation system in place of manual cyanide distillation procedures to determine total cyanide. Strongly-bound cyanide complexes (excluding thiocyanate) are degraded into free cyanide by irradiating the sample in a glass coil. The free cyanide is distilled from the sample matrix and detected using an on-line colorimeter. The concentration of dissociable cyanide complexes is determined by omitting the UV-irradiation step. Thiocyanate can also be determined by using a glass irradiation coil instead of a quartz coil.

Special Considerations: The method was evaluated under EPA's Alternate Test Procedure (ATP) program and can be used in place of ISMO2.3 to prepare and analyze aqueous samples for total cyanide.

Source: U.S. EPA. 2001. "Method Kelada-01: Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate." Washington, DC: U.S. EPA. EPA-821-B-01-009. http://webapp1.dlib.indiana.edu/virtual_disk_library/index.cgi/5315321/FID2672/kelada.pdf

5.2.60 EPA SOP L-A-309: Standard Operating Procedure for Determination of Fentanyl and Carfentanil Oxalate on Wipes Samples By LC/MS/MS

Analyte(s)	CAS RN
Carfentanil	59708-52-0
Fentanyl	437-38-7
3-Methyl fentanyl	42045-87-4

Analysis Purpose: Sample preparation and analyte determination and measurement
Sample Preparation Technique: Shaker extraction followed by filtration using a syringe-PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Carfentanil and fentanyl in wipes
Method Selected for: This method has been selected along with SOP L-A-310 (Section 5.2.61) as options for preparation and analysis of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for carfentanil and fentanyl are 0.43 and 0.48 ng/cm^2, respectively. The reporting range for both compounds is 0.5 – 10.0 ng/cm^2.

Description of Method: Wipes are used to collect samples from surfaces, placed in VOA-vials or similar containers, stored at 0 – 6 °C and analyzed within 24 hours or as soon as possible after collection. A 50-µL aliquot of a surrogate standard solution and 10 mL of acetonitrile are added to each VOA vial, and the wipes are extracted using a shaker table for 15 minutes. The extract is then passed through a syringe filter unit into an autosampler vial and analyzed directly by LC-MS-MS. The LC is operated in hydrophilic interaction liquid chromatography (HILIC) mode, and the ions are transferred into the gas phase using electrospray. The MS is operated in the ESI+ mode. Each target compound is separated and identified by retention time and by comparing the sample primary MRM transition to the standard MRM transition from reference spectra under identical LC-MS-MS conditions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. The concentration of each analyte is determined by instrumentation software using external calibration.

Special Considerations: This procedure was single-laboratory tested by measuring percent recovery and percent RSD in the analytical results of four samples, each consisting of a wipe spiked with 500 ng of carfentanil and 500 ng of fentanyl and processed and analyzed using the method procedures. The average percent recovery was 75 for carfentanil and 87 for fentanyl; RSDs were 11.4 % for carfentanil and 12.8% for fentanyl. 3-Methyl fentanyl was not evaluated.

Source: CSS/PHILIS. 2020. "Standard Operating Procedure for Determination of Fentanyl and Carfentanil Oxalate on Wipes Samples By LC/MS/MS" CSS SOP L-A-309 Rev. 0. Copies of this analytical protocol may be requested from CESER at https://www.epa.gov/esam/forms/contact-us-about-environmental-sampling-analytical-methods-esam-program.

5.2.61 EPA SOP L-A-310: Standard Operating Procedure for Opioids on Wipes by ALTIS UPLC/MS/MS

Analyte(s)	CAS RN
Carfentanil	59708-52-0
Fentanyl	437-38-7
3-Methyl fentanyl	42045-87-4

Analysis Purpose: Sample preparation and analyte determination and measurement

Sample Preparation Technique: Shaker extraction followed by filtration using a syringe-PVDF filter unit
Determinative Technique: Ultra Performance (UP)LC-MS-MS

Method Developed for: Fentanyl and other opiates in wipes
Method Selected for: This method has been selected along with SOP L-A-309 (Section 5.2.60) as options for preparation and analysis of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs are reported for fentanyl (0.176 ng/wipe) and fentanyl-d_5 (0.20 ng/wipe). The reporting range for both compounds is 0.25 – 10.0 ng/cm^2.

Description of Method: Wipes are used to collect samples from surfaces, placed in VOA-vials or similar containers, stored at 0 – 6 °C and analyzed within 24 hours or as soon as possible after collection. A known concentration of surrogate is added, along with 15 mL of methanol as the extraction solvent. The vial is capped and extracted using a shaker table for 15 minutes at 1,500 rpm. The resulting supernatant is decanted into a 25-mL syringe and pressed through a PVDF filter into a graduated cylinder, then diluted to 15 mL with optima grade water. Extract aliquots are transfer to an autosampler vial for direct injection into the UPLC-MS-MS. The UPLC is run using reverse phase chromatography and the ions are transferred into the gas phase using electrospray. The MS is operated in the positive mode (ESI+). Target compounds are separated and identified by retention time and by comparing the sample primary multiple reaction monitoring (MRM) transition to the standard MRM transition from reference spectra under identical LC-MS-MS conditions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. The concentration of each analyte is determined by instrumentation software using external calibration.

Special Considerations: This procedure was developed and tested in a single laboratory, specifically for detection and measurement of fentanyl in wipe samples. Laboratory precision and recovery data are not provided.

Source: CSS/PHILIS. 2021. "Standard Operating Procedure for Opioids on Wipes by ATLIS UPLC/MS/MS" PHILIS SOP L-A-310 Rev. 1. Copies of this analytical protocol may be requested from CESER at https://www.epa.gov/esam/forms/contact-us-about-environmental-sampling-analytical-methods-esam-program.

5.2.62 EPA SOP L-A-507: Analysis of FGAs by GC/MS TOF

Analyte(s)	CAS RN
A-230 (Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate)	2387496-12-8
A-232 (Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-04-8
A-234 (Ethyl N-[(1E)-1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-06-0

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS TOF
Sample Preparation Method: EPA SOP L-P-107 (See Section 5.2.63)
Sample Preparation Technique: Microscale extraction

Method Developed for: A-230, A-232 and A-234 in solid, wipe and water samples

Method Selected for: This method has been selected for the determination and measurement of A-230, A-232 and A-234 in water, solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs are reported as 3.2 ng/wipe (A-230), 0.95 ng/wipe (A-232) and 1.2 ng/wipe (A-234). MDLs are not reported for water or soil samples. The calibration range using standard solutions is reported as 2.5 – 200 pg/µL.

Description of Method: Sample extracts are prepared by microscale extraction following the procedures described in EPA SOP L-P-107 (Section 5.2.63). Internal standards are added to each sample extract for a concentration of 10 ng/mL just prior to analysis.

Special Considerations: This method was developed and tested in a single laboratory. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: PHILIS. 2021. "Standard Operating Procedure for Analysis of FGAs by GCMS TOF" SOP L-A-507, Rev. 3. Copies of this analytical protocol may be requested from CESER at https://www.epa.gov/esam/forms/contact-us-about-environmental-sampling-analytical-methods-esam-program.

5.2.63 EPA SOP L-P-107: Sample Preparation for Chemical Warfare Agent Analysis

Analyte(s)	CAS RN
A-230 (Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate)	2387496-12-8
A-232 (Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-04-8
A-234 (Ethyl N-[(1E)-1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-06-0

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microscale Extraction
Determinative Technique: GC-MS TOF
Determinative Method: EPA SOP L-A-507 (Section 5.2.62).

Method Developed for: GF, HD, GB, GD, A-230, A-232 and A-234 in aqueous, solid, air and wipe samples
Method Selected for: This method has been selected for preparation of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: Surrogates are added to aqueous, wipe and soil samples prior to extraction. Aqueous and wipe samples are extracted with methylene chloride and shaking by hand or using a Vortex mixer, shaker table or sonic bath. Soil samples are extracted with methylene chloride or tris (hydroxymethyl) aminomethane (Tris-buffer) and agitated using a shaker table or sonic bath. All extracts are dried with sodium sulfate, and internal standards are added prior to analysis by GC-MS (see Section 5.2.62).

Special Considerations: The SOP includes two extraction procedures for soil samples. Based on compound similarities, the extraction procedure specified for HD and G-agents is likely the most appropriate procedure for A-230, A-232 and A-234 extraction. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: CSS/PHILIS. 2021. "Standard Operating Procedure for Sample Preparation for Chemical Warfare Agent Analysis" CSS SOP L-P-107 Rev. 3. Copies of this analytical protocol may be requested from CESER at https://www.epa.gov/esam/forms/contact-us-about-environmental-sampling-analytical-methods-esam-program.

5.2.64 NIOSH Method 1612: Propylene Oxide

Analyte(s)	CAS RN
Propylene oxide	75-56-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Coconut shell charcoal solid sorbent tube
Determinative Technique: GC-FID

Method Developed for: Propylene oxide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address propylene oxide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range is 8 – 295 ppm for air samples of 5 L.

Description of Method: A sample tube containing coconut shell charcoal is used for sample collection with a flow rate of 0.01 to 0.2 L/minute. A 1-mL volume of carbon disulfide is added to the vial and allowed to sit for 30 minutes prior to analysis with occasional agitation. Analysis is performed on a GC-FID.

Special Considerations: No interferences have been found. The presence of propylene oxide should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 1612: Propylene Oxide," Issue 2. *NIOSH Manual of Analytical Methods*, 4th Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-1612.pdf

5.2.65 NIOSH Method 2016: Formaldehyde

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: HPLC-UV

Method Developed for: Formaldehyde in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address formaldehyde. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit for formaldehyde is 0.07 µg/sample. The working range is 0.015–to 2.5 mg/m^3 (0.012–2.0 ppm) for a 15-L sample.

Description of Method: This method can be used for the determination of formaldehyde using HPLC with a UV detector. Air is sampled onto a cartridge containing silica gel coated with 2,4-DNPH, at a rate of 0.03 to 1.5 L/minute. The cartridge is extracted with 10 mL of acetonitrile and analyzed by HPLC-UV at a wavelength of 360 nm.

Special Considerations: Ozone has been observed to consume the 2,4-DNPH reagent and to degrade

the formaldehyde derivative. Ketones and other aldehydes can react with 2,4-DNPH; the derivatives produced, however, are separated chromatographically from the formaldehyde derivative.

Source: NIOSH. 2003. "Method 2016: Formaldehyde," Issue 2. *NIOSH Manual of Analytical Methods, Third Supplement*. DHHS (NIOSH) Publication No. 2003-154. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-2016.pdf

5.2.66 NIOSH Method 2513: Ethylene Chlorohydrin

Analyte(s)	CAS RN
2-Chloroethanol	107-07-3
2-Fluoroethanol	371-62-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-FID

Method Developed for: Ethylene chlorohydrin (2-chloroethanol) in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.5–15 ppm for a 20-L air sample.

Description of Method: Samples are drawn into a tube containing petroleum charcoal at a rate of 0.01 to 0.2 L/minute and transferred into vials containing eluent (carbon disulfide, 2-propanol and *n*-pentadiene as an internal standard). Vials must sit for 30 minutes prior to analysis by GC-FID.

Special Considerations: No interferences have been identified. Humidity may decrease the breakthrough volume during sample collection. The presence of 2-chloroethanol should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 2513: Ethylene Chlorohydrin," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-2513.pdf

5.2.67 NIOSH Method 3509: Aminoethanol Compounds II

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected with an impinger containing 15 mL of 2 mM hexanesulfonic acid
Determinative Technique: IC with conductivity detection

Method Developed for: Triethanolamine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD and LOQ for triethanolamine are 0.067 and 0.2 µg/L, respectively.

Description of Method: Samples are collected into an impinger containing 15 mL of 2 mM hexanesulfonic acid using a sampling pump, at a flow rate 0.5 to 1 L/minute for a total sample size of 5 to 300 L. After sampling, the impinger is filled to the 15-mL mark with distilled water and transferred to a vial for shipment. A portion of the sample is filtered through an in-line membrane filter into an autosampler vial. The autosampler injects 50 µL of sample into an ion chromatograph equipped with an ion-pairing guard, cation separator and cation suppressor. Conductivity is set at 3 µS full scale and the eluent used is 2 mM hexanesulfonic acid.

Special Considerations: If high sample throughput is needed, 2 mM hexanesulfonic acid/0.5% v/v acetonitrile can be used as the eluent to reduce run time.

Source: NIOSH. 1994. "Method 3509: Aminoethanol Compounds II," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/3509.pdf

5.2.68 NIOSH Method 3510: Monomethylhydrazine

Analyte(s)	CAS RN
Methyl hydrazine (monomethylhydrazine)	60-34-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected into a bubbler containing hydrochloric acid.
Determinative Technique: Visible spectrophotometry

Method Developed for: Monomethylhydrazine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methyl hydrazine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.027–2.7 ppm for a 20-L sample.

Description of Method: Samples are collected into a bubbler containing hydrochloric acid, using a flow rate of 0.5 to 1.5 L/minute, then transferred to a 25-mL flask, mixed with phosphomolybdic acid solution, diluted with 0.1 M hydrochloric acid, and transferred to a large test tube for spectrophotometric analysis.

Special Considerations: Positive interferences include other hydrazines, as well as stannous and ferrous ion, zinc, sulfur dioxide and hydrogen sulfide. Negative interferences may occur by oxidation of mono-methylhydrazine by halogens, oxygen (especially in the presence of copper (I) ions) and hydrogen dioxide.

Source: NIOSH. 1994. "Method 3510: Monomethylhydrazine," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-3510.pdf

5.2.69 NIOSH Method 5600: Organophosphorus Pesticides

Analyte(s)	CAS RN
Disulfoton	298-04-4
Disulfoton sulfone oxon	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon	2496-92-6
The following analyte should be prepared by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-flame photometric detector (FPD)

Method Developed for: Organophosphorus pesticides in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit depends on the compound being measured. The working range for each analyte is provided in Table 5 of the method. These ranges cover from 0.1 to 2 times the OSHA Permissible Exposure Limits (PELs).

Description of Method: This method is used for the detection of organophosphorus pesticides using a GC-FPD. Samples are prepared by desorbing the sampler resin with 2 mL of toluene/acetone (90/10 v/v) solution. The method also may be applicable to the determination of other organophosphorus compounds after evaluation for desorption efficiency, sample capacity, sample stability, and precision and accuracy. The method also is applicable to Short Term Exposure Limit (STEL) measurements using 12-L samples.

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Several organophosphates may co-elute with either target analytes or internal standards causing integration errors. These include other pesticides, and the following: tributyl phosphate, tris-(2-butoxy ethyl) phosphate, tricresyl phosphate and triphenyl phosphate. The presence of the analytes listed in the table above should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 5600: Organophosphorus Pesticides," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-5600.pdf

5.2.70 NIOSH Method 5601: Organonitrogen Pesticides

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-UV

Method Developed for: Organonitrogen pesticides in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports detection limits of 1.2 µg for aldicarb and 0.6 µg for carbofuran, methomyl and oxamyl for collected air sample volumes of 240 L. The working ranges for aldicarb, carbofuran and oxamyl are listed in Table 2 of the method, and range from 0.5 to 10 times the OSHA PEL.

Description of Method: This method can be used for the determination of organonitrogen pesticides using HPLC with a UV detector. Samples are prepared by desorbing the sampler resin with 2 mL of triethylamine-phosphate solution, rotating end-over-end for 45 minutes, and filtering. The method also may be applicable to the determination of other organonitrogen compounds and to a broad range of pesticides having UV chromophores, e.g., acetanilides, acid herbicides, organophosphates, phenols, pyrethroids, sulfonyl ureas, sulfonamides, triazines and uracil pesticides.

Special Considerations: The presence of analytes listed in the table above should be confirmed using either a secondary HPLC column or an MS. Because of the broad response of the UV detector at shorter wavelengths, there are many potential interferences. Those tested include solvents (chloroform and toluene), antioxidants (butylated hydroxytoluene [BHT]), plasticizers (dialkyl phthalates), nitrogen compounds (nicotine, caffeine), HPLC reagent impurities (e.g., in triethylamine), other pesticides (2,4-dichlorophenoxyacetic acid [2,4-D], atrazine, parathion), and pesticide hydrolysis products (1-naphthol).

Source: NIOSH. 1998. "Method 5601: Organonitrogen Pesticides," Issue 1. *NIOSH Manual of Analytical Methods, Second Supplement*. DHHS (NIOSH) Publication No. 98-119. Washington, DC: DHHS (NIOSH). http://www.cdc.gov/niosh/docs/2003-154/pdfs/5601.pdf

5.2.71 NIOSH Method 6001: Arsine

Analyte(s)	CAS RN
Arsine	7784-42-1

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Coconut shell charcoal solid sorbent tube
Determinative Technique: Graphite furnace atomic absorption (GFAA)

Method Developed for: Arsine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address arsine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.001–0.2 mg/m^3 for a 10-L sample.

Description of Method: Arsine is determined as arsenic. 0.1 to 10 L of air is drawn through a sorbent tube containing activated charcoal. The sorbent is extracted with a nitric acid solution, and arsenic is determined by GFAA.

Special Considerations: The method is subject to interferences from other arsenic compounds.

Source: NIOSH. 1994. "Method 6001: Arsine," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6001.pdf

5.2.72 NIOSH Method 6002: Phosphine

Analyte(s)	CAS RN
Phosphine	7803-51-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption with hot acidic potassium permanganate solution
Determinative Technique: Visible spectrophotometry

Method Developed for: Phosphine in air

Method Selected for: This method has been selected for preparation and analysis of air samples to address phosphine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.02–0.9 mg/m^3 for a 16-L sample.

Description of Method: Phosphine is determined as phosphate. 1 to 16 L of air is drawn through a sorbent tube containing silica gel coated with mercuric cyanide. The sorbent is extracted with a potassium permanganate/sulfuric acid solution and washed with reagent water. Following treatment with the color agent and extraction into organic solvent, phosphate is determined by visible spectrometry.

Special Considerations: The method is subject to interferences from phosphorus trichloride, phosphorus pentachloride and organic phosphorus compounds.

Source: NIOSH. 1998. "Method 6002: Phosphine," Issue 2. *NIOSH Manual of Analytical Methods, Second Supplement*. DHHS (NIOSH) Publication No. 98-119. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6002.pdf

5.2.73 NIOSH Method 6010: Hydrogen Cyanide

Analyte(s)	CAS RN
Cyanide, Total	57-12-5
Hydrogen cyanide	74-90-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: Visible spectrophotometry

Method Developed for: Hydrogen cyanide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 3–260 mg/m^3 for a 3-L sample.

Description of Method: Hydrogen cyanide is determined as a cyanide ion complex by this method. A volume of 0.6 to 90 L of air is drawn through a soda lime sorbent tube. A glass-fiber filter is used to remove particulate cyanides prior to the sorbent tube. Cyanide is extracted from the sorbent with reagent water treated with sodium hydroxide. The extract is pH adjusted with hydrochloric acid, oxidized with N-chlorosuccinimide/succinimide, and treated with the coupling-color agent (barbituric acid/pyridine). The cyanide ion is determined by visible spectrophotometry using a wavelength of 580 nm.

Special Considerations: The method is subject to interference from high concentrations of hydrogen sulfide. Two liters is the minimum volume required to measure concentration of 5 ppm.

Source: NIOSH. 1994. "Method 6010: Hydrogen Cyanide," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6010.pdf

5.2.74 NIOSH Method 6013: Hydrogen Sulfide

Analyte(s)	CAS RN
Hydrogen sulfide	7783-06-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction

Determinative Technique: IC with conductivity detection

Method Developed for: Hydrogen sulfide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen sulfide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.9–20 mg/m^3 for a 20-L sample.

Description of Method: This method determines hydrogen sulfide as sulfate. 15 to 40 L of air is drawn through charcoal sorbent. A prefilter is used to remove particulates. The sorbent portions are extracted with an ammonium hydroxide/hydrogen peroxide solution and the extract is analyzed for sulfate by IC.

Special Considerations: The method is subject to interference from sulfur dioxide.

Source: NIOSH. 1994. "Method 6013: Hydrogen Sulfide," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6013.pdf

5.2.75 NIOSH Method 6016: Ammonia

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction
Determinative Technique: Ion chromatography

Method Developed for: Ammonia in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 17 – 68 mg/m^3 for a 30-L sample.

Description of Method: Ammonia is determined as ammonium ion by this method. A volume of 0.1 to 96 L of air is drawn through a sulfuric acid-treated silica gel sorbent. A prefilter is used to remove particulates. The sorbent is extracted with reagent water, then the extract is transferred to autosampler vials using a syringe with inline filter and analyzed by ion chromatography with conductivity detection.

Special Considerations: Ethanolamines (monoethanolamine, isopropylamine, and propanolamine) have retention times similar to ammonium ion. The use of the weak (alternate) eluent described in the method will aid in separating these peaks.

Source: NIOSH. 1996. "Method 6016: Ammonia," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
https://www.cdc.gov/niosh/docs/2003-154/pdfs/6016.pdf

5.2.76 NIOSH Method 6402: Phosphorus Trichloride

Analyte(s)	CAS RN
Phosphorus trichloride	7719-12-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Add reagent to samples in bubbler solution and heat
Determinative Technique: Visible spectrophotometry

Method Developed for: Phosphorus trichloride in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address phosphorus trichloride. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 1.2–80 mg/m^3 for a 25-L sample.

Description of Method: In this method, phosphorus trichloride is determined as phosphate. A volume of 11 to 100 L of air is drawn through a bubbler containing reagent water. The resulting phosphorus acid solution is oxidized with bromine to phosphoric acid and color agent (sodium molybdate) and reducing agent (hydrazine sulfate) are added. The solution is analyzed for the resulting molybdenum blue complex by visible spectrophotometry.

Special Considerations: Phosphorus (III) compounds can interfere with analysis of phosphorus trichloride, by increasing the amount of phosphorus that is measured. Phosphorus (V) compounds do not interfere.

Source: NIOSH. 1994. "Method 6402: Phosphorus Trichloride," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6402.pdf

5.2.77 NIOSH Method 7905: Phosphorus

Analyte(s)	CAS RN
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: GC solid sorbent tube and solvent extracted (desorbed)
Determinative Technique: GC-FPD

Method Developed for: Phosphorus in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address white phosphorus. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The LOD for samples analyzed by GC-FPD is 0.005 µg per sample. The working range for samples analyzed by GC-FPD is 0.056–0.24 mg/m^3 for a 12-L sample.

Description of Method: This method identifies and determines the concentration of white phosphorus in air by using a GC-FPD. Five to 100 L of air is drawn through a GC solid sorbent tube, and the sorbent is extracted (desorbed) with xylene. The method is applicable to vapor-phase phosphorus only; if particulate phosphorus is expected, a filter can be used in the sampling train.

Special Considerations: The presence of white phosphorus should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 7905: Phosphorus," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-7905.pdf

5.2.78 NIOSH Method 7906: Particulate Fluorides and Hydrofluoric Acid by Ion Chromatography

Analyte(s)	CAS RN
Hydrogen fluoride	7664-39-3

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Aqueous solution extraction
Determinative Technique: IC with conductivity detection

Method Developed for: Fluorides in aerosol and gas
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen fluoride. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.04–8 mg/m^3 for 250-L samples.

Description of Method: Hydrogen fluoride is determined as fluoride ion by this method. A volume of 15 to 1,000 L of air is drawn through a 0.8-µm cellulose nitrate prefilter (to trap particulate fluorides) and a cellulose nitrate filter treated with sodium carbonate (to trap gaseous fluoride). The filter is extracted with an aqueous solution of 8 mM sodium carbonate /1 mM sodium bicarbonate and the extract is analyzed for fluoride by IC.

Special Considerations: If other aerosols are present, gaseous fluoride may be slightly underestimated due to adsorption onto or reaction with particles, with concurrent overestimation of particulate/gaseous fluoride ratio.

Source: NIOSH. 2014. "Method 7906: Particulate Fluorides and Hydrofluoric Acid 7906 by Ion Chromatography," Issue 2. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/7906.pdf

5.2.79 NIOSH Method 7907: Volatile Acids by Ion Chromatography (Hydrogen Chloride, Hydrogen Bromide, Nitric Acid)

Analyte(s)	CAS RN
Hydrogen bromide	10035-10-6
Hydrogen chloride	7647-01-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Aqueous solution extraction
Determinative Technique: IC with conductivity detection

Method Developed for: Hydrogen bromide, hydrogen chloride and nitric acid in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen bromide and hydrogen chloride. See Appendix A for the corresponding method usability tiers.
Detection and Quantitation: The working range is 0.04–8 mg/m^3 for hydrogen bromide and hydrogen chloride in 240-L samples.

Description of Method: Hydrogen bromide and hydrogen chloride are determined as bromide and chloride ions, respectively. A volume of 30 to 600 L of air is drawn through a 37-mm diameter quartz fiber prefilter (to trap potentially interfering particulate chlorides) and a 37-mm diameter quartz fiber filter treated with sodium carbonate (to trap gaseous hydrogen bromide and hydrogen chloride). After discarding the prefilters, the filter is extracted with an aqueous solution of 3.1 mM sodium carbonate/0.35 mM sodium carbonate and the extract is analyzed for bromide and chloride by IC.

Special Considerations: Inorganic acids can react with co-sampled particulate matter on the pre-filter, leading to low results (e.g., zinc oxide reacting with hydrochloric acid). Potentially interfering particulate chlorides and nitrates removed by the pre-filter can react with the sampled acids and liberate hydrochloric acid that is subsequently collected on the sampling filter, leading to high results. Silica gel sorbent tubes

can be used instead of treated filters, but each sorbent tube must be preceded by a pre-filter.

Source: NIOSH. 2014. "Method 7907: Volatile Acids by Ion Chromatography (Hydrogen Chloride, Hydrogen Bromide, Nitric Acid)," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/7907.pdf

5.2.80 NIOSH Method 9102: Elements on Wipes

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
Chlorovinyl arsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Ethyldichloroarsine (ED)	598-14-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: ICP-AES / ICP-MS / Spectrophotometry
Determinative Method: EPA SW-846 Methods 6010D, 6020B, 7473 and 8270E. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Measurement of metals on wipe surfaces using ICP-AES
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above as total arsenic, mercury, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working ranges are: 0.261–105 µg/wipe (arsenic), 0.136–50.0 µg/wipe (thallium), and 0.0333–25.0 µg/wipe (vanadium). A working range is not provided for mercury.

Description of Method: Surface wipe samples are transferred to a clean beaker, followed by the addition of concentrated nitric and perchloric acids. The beaker contents are held at room temperature for 30 minutes, then heated at 150°C for 8 hours. Additional nitric acid is added until the wipe media is completely destroyed. The sample is then taken to near dryness and the residue dissolved and diluted before being analyzed.

Special Considerations: ICP-MS may also be used for the analysis of wipe samples; however, at this time, this technique has not been evaluated for wipes. Nitric and perchloric acids are strong oxidizers and

extremely corrosive. Perform all perchloric acid digestions in a perchloric acid hood. When working with acids, use gloves and avoid inhalation or contact with skin or clothing. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: NIOSH. 2003. "Method 9102, Issue 1: Elements on Wipes." *NIOSH Manual of Analytical Methods*, 3rd Supplement 2003-154. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-9102.pdf

5.2.81 NIOSH Method 9106: Methamphetamine and Illicit Drugs, Precursors and Adulterants on Wipes by Liquid-Liquid Extraction

Analyte(s)	CAS RN
Phencyclidine	77-10-1

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS

Method Developed for: Phencyclidine in wipe samples
Method Selected for: This method has been selected for the preparation and analysis of wipe samples to address phencyclidine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDLs for phencyclidine on spiked cotton gauze in full-scan and SIM mode are 0.3 and 0.2 ng/cm^2, respectively. The working range of the method is 1–100 ng/ cm^2 for both full-scan and SIM modes.

Description of Method: Internal standards and desorption solution (0.1M sulfuric acid) are added to each sample contained in a centrifuge tube. The tubes are capped and the samples and solution are mixed with a rotatory mixer at 10–30 rpm for at least one hour. If necessary, the pH is adjusted to ≤4 with 3M sulfuric acid. Sample extracts are transferred to a glass centrifuge tube and cleaned by adding 10 mL of hexane to 10 mL of extract. The contents are mixed by rotary mixer for at least one hour, then allowed to stand for 15–20 minutes. If an emulsion forms, extracts are centrifuged for a few minutes at 1,500–2,000 rpm. The upper organic layer is aspirated off as waste, and 1–2 drops of pH indicator (phenolphthalein and bromothymol blue) and 0.5 mL of 10M sodium hydroxide are added to the aqueous fraction, as needed, to turn the solution to purple or magenta. Once this color change is achieved, 10 mL of methylene chloride is added, the sample container is capped and the contents remixed on a rotary mixer for 1 hour. The mixture is allowed to stand for 15–30 minutes, and the centrifuge procedure is repeated if an emulsion forms. Any remaining water is removed using packed potassium carbonate-sodium sulfate drying columns. The methylene chloride is evaporated under nitrogen, and 100 µL of chlorodifluoroacetic anhydride is added followed by additional mixing. The tube is then placed into an oven at 70–75°C and heated for 20–30 minutes. After cooling, the extract is evaporated to dryness under nitrogen until a blue or violet color is visible. Reconstituting solution (1 mL) is added and the solution is transferred to an amber-colored GC vial containing 200–250 mg anhydrous sodium sulfate. Vials are capped and analyzed by GC-MS.

Special Considerations: If an oil-like residue or film persists, the sample may contain contaminants that were not removed during the cleanup step or were introduced following sample cleanup. In such cases, the cleanup step is repeated on another 10-mL sample aliquot, using methylene chloride instead of hexane as the cleanup solvent. Analyte losses have been experienced during the derivatization step if blowing is continued for more than 2 minutes beyond the appearance of a blue or violet color.

Source: NIOSH. 2011. "Method 9106: Methamphetamine and Illicit Drugs, Precursors and Adulterants on Wipes by Liquid-Liquid Extraction," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition.

Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/9106.pdf

5.2.82 NIOSH Method 9109: Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction

Analyte(s)	CAS RN
Phencyclidine	77-10-1

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS

Method Developed for: Phencyclidine in wipe samples
Method Selected for: This method has been selected for preparation and analysis of wipe samples to address phencyclidine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDLs for phencyclidine on spiked cotton gauze and AlphaWipes wipes (All-Spec, Wilmington, NC, or equivalent) are 1 and 5 ng/cm^2, respectively. The working range of the method is 3–300 ng/ cm^2.

Description of Method: Internal standards and desorption solution (0.1 M sulfuric acid) are added to wipe samples contained in a centrifuge tube. The tubes are capped and samples mixed with a rotatory mixer at 10–30 rpm for at least one hour. If necessary, the pH is adjusted to ≤4 with 2.5 to 3M sulfuric acid. An SPE column is attached to a vacuum capable of 25–30 psi pressure and conditioned with 3 mL of methanol, followed by 1 mL of ASTM Type II deionized water. The SPE column is loaded with 10 mL of sample and the sample is pulled through the column via vacuum. The column is then washed with 3 mL of 0.1M hydrochloric acid, followed by 3 mL of methanol, and all effluent is discarded. The vacuum is increased to remove all traces of water, and the analytes are eluted with 3 mL of 80:20:2 methylene chloride:isopropanol:concentrated ammonium hydroxide (v/v) into a collection tube. About 5 µL of crystal violet solution and 100 µL of 0.3M hydrochloric acid in methanol are added to the tube, and the samples are evaporated to dryness under nitrogen. Acetonitrile containing internal standard (100 µL) and derivatizing agents is added to the collection tube, and the tubes are capped, vortexed for 4–5 seconds, and a 300–500 µL aliquot is transferred to an autosampler vial for analysis by GC-MS.

Special Considerations: No chromatographic interferences were observed during method development; however, water, surfactants and polyols can inhibit derivatization. The color of the reconstituted solution should be deep blue to violet. If the color turns light blue or turquoise upon standing, moisture may be present. Such samples need to be reprocessed beginning at the SPE extraction step, since the derivatives are not stable in the presence of moisture.

Source: NIOSH. 2011. "Method 9109: Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). http://www.cdc.gov/niosh/docs/2003-154/pdfs/9109.pdf

5.2.83 NIOSH Method S301-1: Fluoroacetate Anion

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts	NA
Methyl fluoroacetate	453-18-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction
Determinative Technique: LC-MS

Determinative Method: Adapted from J. Chromatogr. A, 1139 (2002) 271-278.

Method Developed for: Fluoroacetate anion in air
Method Selected for: This method has been selected for preparation of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit is estimated to be 20 ng of sodium fluoroacetate per injection, corresponding to a 100-µL aliquot of a 0.2-µg/mL standard. The analytical range of this method is estimated to be 0.01–0.16 mg/m^3.

Description of Method: This method was developed specifically for sodium fluoroacetate, but also may be applicable to other fluoroacetate salts. The method determines fluoroacetate salts as fluoroacetate anion. A known volume of air (e.g., 480 L was used in validation of this method) is drawn through a cellulose ester membrane filter to collect sodium fluoroacetate. Sodium fluoroacetate is extracted from the filter with 5 mL of deionized water, and the resulting sample is analyzed by LC-MS.

Special Considerations: When analyzing samples for methyl fluoroacetate (as fluoroacetate ion), addition of base is required to assist dissociation into fluoroacetate anion.

Source: NIOSH. 1977. "Method S301-1: Sodium Fluoroacetate." *NIOSH Manual of Analytical Methods*, Second Edition, Volume 5. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-s301-1.pdf

5.2.84 OSHA Method 40: Methylamine

Analyte(s)	CAS RN
Methylamine	74-89-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-FL/vis

Method Developed for: Methylamine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methylamine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 0.35 µg per sample (28 ppb or 35 µg/m^3). Quantitation limits of 28 ppb (35 µg/m^3) have been achieved. This is the smallest amount of methylamine that can be quantified within the requirements of a recovery of at least 75% and a precision (standard deviation of 1.96) of ± 25% or better.

Description of Method: This method is used for detection of methylamine using HPLC with a FL or visible (vis) detector. Samples are collected by drawing 10-L volumes of air at a rate of 0.2 L/minute through standard size sampling tubes containing sampler resin coated with 10% 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) by weight. Samples are desorbed with 5% (w/v) NBD chloride in tetrahydrofuran (with a small amount of sodium bicarbonate present), heated in a hot water bath, and analyzed by HPLC-FL or high performance liquid chromatography-visible (HPLC-vis).

Source: OSHA. 1982. "Method 40: Methylamine." Method originally obtained from https://www.osha.gov, but is provided here for reference. Salt Lake City, UT: OSHA.
http://www.epa.gov/sites/production/files/2015-07/documents/osha-method40.pdf

5.2.85 OSHA Method 54: Methyl Isocyanate (MIC)

Analyte(s)	CAS RN
Methyl isocyanate	624-83-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC

Method Developed for: Methyl isocyanate in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methyl isocyanate. See Appendix A for the corresponding method usability tier.

Description of Method: This method determines the concentration of methyl isocyanate in air by using HPLC with a FL or UV detector. Samples are collected by drawing a known volume of air through sampler tubes containing resin coated with 0.3 mg of 1-(2-pyridyl)piperazine (1-2PP). Samples are desorbed with acetonitrile and analyzed by HPLC using a FL or UV detector.

Source: OSHA. 1985. "Method 54: Methyl Isocyanate (MIC)." Method originally obtained from https://www.osha.gov, but is provided here for reference. Sandy, UT: OSHA. https://www.osha.gov/sites/default/files/methods/osha54.pdf

5.2.86 OSHA Method 61: Phosgene

Analyte(s)	CAS RN
Phosgene	75-44-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-NPD

Method Developed for: Phosgene in air samples
Method Selected for: This method has been selected for preparation and analysis of air samples to address phosgene. See Appendix A for the corresponding method usability tier.

Description of Method: This method determines the concentration of phosgene in air by using GC with an NPD. Air samples are collected by drawing known volumes of air through sampling tubes containing resin adsorbent that has been coated with 2-(hydroxymethyl)piperidine. The samples are desorbed with toluene and then analyzed by GC using an NPD.

Special Considerations: The presence of phosgene should be confirmed using either a secondary GC column or an MS.

Source: OSHA. 1986. "Method 61: Phosgene." Method originally obtained from https://www.osha.gov, but is provided here for reference. Salt Lake City, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-method61.pdf

5.2.87 OSHA Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres

Analyte(s)	CAS RN
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Buffer desorption
Determinative Technique: IC-UV

Method Developed for: Sodium azide and hydrazoic acid in workplace atmospheres
Method Selected for: This method has been selected for preparation and analysis of air and wipe samples to address sodium azide as azide ion. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for sodium azide was found to be 0.003 mg/m^3 for a 5-L air sample. The quantitation limit was found to 0.011 mg/m^3, also for a 5-L air sample.

Description of Method: This method describes sample collection and analysis of airborne azides (as sodium azide and hydrazoic acid). Particulate sodium azide is collected on a polyvinyl chloride (PVC) filter or in the glass wool plug of the sampling tube. Gaseous hydrazoic acid is collected and converted to sodium azide by the impregnated silica gel (ISG) sorbent within the sampling tube. The collected azide on either media is desorbed in a weak buffer solution, and the resultant azide anion is analyzed by IC using a variable wavelength UV detector at 210 nm. A gravimetric conversion is used to calculate the amount of sodium azide or hydrazoic acid collected.

Source: OSHA. 1992. "Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres." Sandy, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-id-211.pdf

5.2.88 OSHA Method ID216SG: Boron Trifluoride (BF_3)

Analyte(s)	CAS RN
Boron trifluoride	7637-07-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Sample collected in bubbler (no sample preparation required)
Determinative Technique: Ion specific electrode (ISE)

Method Developed for: Boron trifluoride in air samples
Method Selected for: This method has been selected for preparation and analysis of air samples to address boron trifluoride. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 10 µg in a 30-L sample.

Description of Method: Boron trifluoride is determined as fluoroborate. A volume of 30 to 480 L of air is drawn through a bubbler containing 0.1M ammonium fluoride. The bubbler solution is diluted and analyzed with a fluoroborate ISE.

Source: OSHA. 1989. "Method ID216SG: Boron Trifluoride (BF_3)." Method originally obtained from https://www.osha.gov, but is provided here for reference. Sandy, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-id216sg.pdf

5.2.89 OSHA Method PV2004: Acrylamide

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Methyl acrylonitrile	126-98-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-UV

Method Developed for: Acrylamide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit was found to be 0.7 µg/mL (0.006 mg/m^3 for a 1-mL desorption volume) or 0.029 mg/m^3 (for a 5-mL desorption volume), based on a 120-L air sample). Applicable working ranges for 1-mL and 5-mL desorption volumes are 0.017–1.5 mg/m^3 and 0.083–7.5 mg/m^3, respectively.

Description of Method: This method determines the concentration of acrylamide in air by using HPLC with a UV detector. Samples are collected by drawing known volumes of air through OSHA versatile sampler (OVS-7) tubes, each containing a glass fiber filter and two sections of adsorbent. Samples are desorbed with a solution of 5% methanol/95% water, and analyzed by HPLC-UV.

Special Considerations: The presence of acrylamide, acrylonitrile and methyl acrylonitrile should be confirmed using either a secondary HPLC column or an MS.

Source: OSHA. 1991. "Method PV2004: Acrylamide." Sandy, UT: OSHA.
http://www.epa.gov/sites/production/files/2015-07/documents/osha-pv2004.pdf

5.2.90 OSHA Method PV2103: Chloropicrin

Analyte(s)	CAS RN
Chloropicrin	79-06-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-ECD

Method Developed for: Chloropicrin in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address chloropicrin. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 0.01 ng, with a 1-µL injection volume. This is the smallest amount that could be detected under normal operating conditions. The working range is 33.2–1330 µg/m^3.

Description of Method: This method determines the concentration of chloropicrin in air by GC-ECD. Samples are collected by drawing a known volume of air through two adsorbent tubes in series. Samples are desorbed with ethyl acetate and analyzed by GC-ECD.

Special Considerations: The presence of chloropicrin should be confirmed using either a secondary GC column or an MS. Chloropicrin is light sensitive, and samples should be protected from light.

Source: OSHA. 1991. "Method PV2103: Chloropicrin." Salt Lake City, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-pv2103.pdf

5.2.91 ASTM Method D5755-09(e1): Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct transfer
Determinative Technique: Transmission electron microscopy (TEM)

Method Developed for: Asbestos in dust
Method Selected for: This method has been selected for preparation and analysis of solid (e.g., soft surfaces-microvac) samples to address asbestos. See Appendix A for the corresponding method usability tier.

Description of Method: This method describes procedures to identify asbestos in dust and provide an estimate of surface loading reported as the number of asbestos structures per unit area of sampled surface. Samples are collected by vacuuming a known surface area with a standard 25- or 37-mm air sampling cassette using a plastic tube that is attached to the inlet orifice, which acts as a nozzle. Once collected, samples are transferred from inside the cassette to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane, and a section of the membrane is prepared and transferred to a TEM grid using a direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using select area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA) at a magnification of 15,000 to 20,000X.

Source: ASTM. 2014. "Method D5755-09(e1): Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D5755.htm

5.2.92 ASTM Method D6480-19: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct transfer
Determinative Technique: TEM

Method Developed for: Asbestos in samples wiped from surfaces
Method Selected for: This method has been selected for preparation and analysis of wipe (e.g., hard surfaces-wipes) samples to address asbestos. See Appendix A for the corresponding method usability tier.

Description of Method: This method describes a procedure to identify asbestos in samples wiped from surfaces and to provide an estimate of the concentration reported as the number of asbestos structures per unit area of sampled surface. Samples are collected by wiping a surface of known area with a wipe

material. Once collected, samples are transferred from the wipe material to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane filter, and a section of the membrane filter is prepared and transferred to a TEM grid, using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using electron diffraction and EDXA at a magnification from 15,000 to 20,000X.

Source: ASTM. 2019. "Method D6480-19: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D6480.htm

5.2.93 ASTM Method D7597-16: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex-HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IMPA, MPA and PMPA in surface water
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. ***Note***: EPA Method 538 (Section 5.2.11) has been selected for sample preparation and analysis of DIMP in drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection verification levels (DVLs) and reporting range vary for each analyte and range from 0.25 to 20 µg/L and 5 to 1,500 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 1 day. The target compounds are identified by comparing the sample single reaction monitoring (SRM) transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition of the target compounds and external standard calibration.

Special Considerations: Method modifications (e.g., pH adjustment) may be needed when analyzing for dimethyphosphoramidic acid.

Source: ASTM. 2016. "Method D7597-16: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International.

http://www.astm.org/Standards/D7597.htm

5.2.94 ASTM Method D7598-16: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in surface water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address thiodiglycol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL for thiodiglycol is 20 µg/L; the reporting range is 100–10,000 µg/L.

Description of Method: Thiodiglycol is analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 7 days. The target compound is identified by comparing the sample primary SRM transition to the known standard SRM transition. The retention time must fall within the retention time of the standard by ± 5%. Thiodiglycol is quantitated using the primary SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7598-16: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7598.htm

5.2.95 ASTM Method D7599-16: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Diethanolamine, triethanolamine, MDEA and EDEA in surface water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL and reporting range for EDEA and TEA are 5 µg/L and 25–500 µg/L, respectively. The DVL and reporting range for MDEA are 10 µg/L and 50–500 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization by

LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 7 days. Target compounds are identified by comparing sample SRM transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7599-16: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7599.htm

5.2.96 ASTM Method D7644-16: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Diphacinone	82-66-6

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven PVDF filter unit for water samples; automated Soxhlet or pressured fluid extraction for solid samples, and solvent extraction for wipes.
Determinative Technique: LC-MS-MS

Method Developed for: Bromadiolone, brodifacoum and diphacinone in reagent, surface and drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid and wipe samples to address the analytes listed in the table above. ***Note***: EPA SW-846 Methods 3541/3545A (Sections 5.2.22 and 5.2.23) and Methods 3570/8290A Appendix A (Sections 5.2.24 and 5.2.36) have been selected for preparation of solid and wipe samples, respectively. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVLs and reporting range for each analyte are 0.020 µg/L and 0.125–2.5 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization using LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit, and analyzed directly by LC-MS-MS within 14 days. The target analytes are identified by retention time and two SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the primary SRM transition of the analytes and external standard calibration. Analytes are confirmed using the confirmatory SRM transitions.

Source: ASTM. 2016. "Method D7644-16: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7644.htm

5.2.97 ASTM Method D7645-16: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran	1563-66-2
Oxamyl	23135-22-0
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, oxamyl and thiofanox in water
Method Selected for: This method has been selected for preparation and analysis of non-drinking water samples to address aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, oxamyl and thiofanox. It has also been selected for the analysis of prepared solid and wipe samples to address thiofanox. See Appendix A for corresponding method usability tiers. ***Note***:

- SW-846 Methods 3541 (see Section 5.2.22) or 3545A (see Section 5.2.23) have been selected for preparation of solid samples to be analyzed for thiofanox.
- SW-846 Methods 3570 (see Section 5.2.24) and 8290A Appendix A (see Section 5.2.36) have been selected for preparation of wipe samples to be analyzed for thiofanox.

Detection and Quantitation: A DVL is reported as 250 ng/L for all compounds listed in the table above. The reporting range for these compounds is 1–100 µg/L.

Description of Method: Samples are spiked with surrogates, filtered using a syringe-driven filter unit, and analyzed directly by LC-MS-MS within 14 days. Target analytes are identified by comparing primary and confirmatory MRM transitions to known standard primary and confirmatory MRM transitions. The retention time for the analytes must fall within ± 5% of the retention time of the analytes in standard solution. Analytes are measured using the primary SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7645-16: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7645.htm

5.2.98 ASTM Method E2787-11: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using PFE, and filtered using a syringe-driven PVDF filter unit

Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in solid samples
Method Selected for: This method has been selected for preparation and analysis of solid samples to address thiodiglycol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL is 54 µg/kg. The reporting range is 200–16,000 µg/kg.

Description of Method: Approximately 5–30 g of soil is mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using methanol. Extracts are filtered using a 0.2-micron filter and concentrated to a final volume of 0.4 mL using a nitrogen evaporation device. The volume of the extract is brought up to 2 mL with HPLC-grade water and analyzed by LC-MS-MS. The target analytes are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method E2787-11: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/ Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2787.htm

5.2.99 ASTM Method E2838-11: Standard Test Method for Determination of Thiodiglycol on Wipes by Solvent Extraction Followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using sonication or PFE and filtered using a syringe-driven PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in wipes
Method Selected for: This method has been selected for preparation and analysis of wipe samples to address thiodiglycol. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDL is 0.085 µg/wipe. The reporting range is 1–80 µg/wipe.

Description of Method: Wipe samples are shipped to the laboratory at 0 to 6°C, and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Extraction may be performed using sonication or PFE. Extracts are filtered using a 0.2-micron filter and concentrated to a final volume of 2 mL when using sonication or 4 mL when using PFE. If sample throughput is less of a concern, the PFE extracts can be concentrated down to 2 mL. Extracts are analyzed by LC-MS-MS. Thiodiglycol is identified by comparing the SRM transitions to the known standard SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method E2838-11: Standard Test Method for Determination of Thiodiglycol on Wipes by Solvent Extraction Followed by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2838.htm

5.2.100 ASTM Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Soil by Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using PFE and filtered using a syringe-driven Millex-HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IPMA, MPA and PMPA in soil
Method Selected for: This method has been selected for preparation and analysis of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The reporting range for all analytes is 40–2,000 µg/kg. MDLs range from 1.3 to 8.7 µg/kg.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Approximately 5–30 g of soil are mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using water. Extracts are filtered using a 0.2-micron filter and analyzed by LC-MS-MS. The target compounds are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition of the target compounds and external standard calibration.

Special Considerations: Method modifications (e.g., pH adjustment) may be needed when analyzing for dimethyphosphoramidic acid.

Source: ASTM. 2016. "Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Soil by Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2866.htm

5.2.101 ISO Method 10312:1995: Ambient Air - Determination of Asbestos Fibres - Direct-Transfer Transmission Electron Microscopy Method

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement

Sample Preparation Technique: Direct transfer
Determinative Technique: TEM

Method Developed for: Asbestos in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to address asbestos. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: In a 4,000-L air sample with approximately 10 pg/m^3 (typical of clean or rural atmospheres), an analytical sensitivity of 0.5 structure/L can be obtained. This is equivalent to a detection limit of 1.8 structure/L when an area of 0.195 mm of the TEM specimen is examined. The range of concentrations that can be determined is 50–7,000 $structures/mm^2$ on the filter.

Description of Method: This method determines the type(s) of asbestos fibers present, but cannot discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral. The method is defined for polycarbonate capillan/pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

Source: ISO. 2005. "Method 10312: 1995: Ambient Air - Determination of Asbestos Fibres - Direct Transfer Transmission Electron Microscopy Method."
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=18358

5.2.102 Standard Method 4500-CN G: Cyanides Amenable to Chlorination after Distillation

Analyte(s)	CAS RN
Cyanide, Amenable to chlorination	NA

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion followed by distillation
Determinative Technique: Visible spectrophotometry, titrimetry or cyanide-selective electrode

Method Developed for: Cyanide in drinking water, ground water, surface water, domestic and industrial wastewaters, and solid waste
Method Selected for: This method has been selected for preparation and analysis of water samples to address cyanide amenable to chlorination, as an alternative to EPA Regional Laboratory (RLAB) Method 3135.2I (see Section 5.2.42), for use by laboratories more familiar with its procedures.
Detection and Quantitation: The method has been evaluated in the ranges of 0.008–0.191 mg/L (colorimetric procedure) and 1–4 mg/L (titrimetric procedure). These ranges can be expanded by sample dilution, by either distilling less sample or diluting the distillate.

Description of Method: This method is applicable to the determination of cyanides amenable to chlorination (also known as available cyanide). After part of the sample is chlorinated to decompose the cyanides, both the chlorinated and the untreated samples are distilled as described in Standard Method (SM) 4500-CN C. The difference between the cyanide concentrations in the two samples is expressed as cyanides amenable to chlorination. The sample is divided into two equal portions of 500 mL (or equal portions diluted to 500 mL). Chlorinate one of the portions using the procedure in the next paragraph. Both portions are analyzed for cyanide and the difference in determined concentrations is the cyanide amenable to chlorination.

One portion is placed in a 1-L beaker covered with aluminum foil or black paper. The beaker is kept covered with a wrapped watch glass during chlorination. Calcium hypochlorite solution is added dropwise to the sample while agitating and maintaining pH between 11 and 12 by adding sodium hydroxide solution. The sample is then tested for chlorine by placing a drop of treated sample on a strip of KI-starch

paper. A distinct blue color indicates sufficient chlorine (approximately 50 to 100 mg chlorine/L). The sample is agitated for 1 hour, while adding more calcium hypochlorite if necessary to maintain the chlorine concentration. After agitating for 1 hour, residual chlorine is removed by the dropwise addition of sodium arsenite solution (2g/100 mL) or by the addition of 8 drops of hydrogen peroxide (3% solution) followed by 4 drops of sodium thiosulfate (500 g/L). The sample is tested with potassium iodide-starch paper by adding a drop or two of sample to the paper. The dechlorinating solutions are to be added until there is no color change. Both the chlorinated and unchlorinated samples are distilled as described in 4500-CN C. The samples are tested according to the procedures in SM 4500-CN D (titrimetric), E (colorimetric) or F (cyanide-selective electrode).

Special Considerations: Samples should be protected from exposure to UV radiation. All sample manipulations should be performed under incandescent light, to prevent photodecomposition of some metal-cyanide complexes by UV light. Some unidentified organic chemicals may oxidize or form breakdown products during chlorination, giving higher results for cyanide after chlorination than before chlorination. This may lead to a negative value for cyanides amenable to chlorination after distillation for wastes from, for example, the steel industry, petroleum refining, and pulp and paper processing. Where such interferences are encountered use SM 4500-CN I for determining dissociable cyanide.

Source: APHA, AWWA and WEF. 2017. "Method 4500-CN G: Cyanides Amenable to Chlorination after Distillation." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.103 Standard Method 4500-NH_3 B: Nitrogen (Ammonia) Preliminary Distillation Step

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation
Sample Preparation Technique: Distillation
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-NH_3 G

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or ground water, and good-quality nitrified wastewater effluent
Method Selected for: This method has been selected for preparation of non-drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.

Description of Method: A 0.5- to 1-L sample is dechlorinated, buffered, adjusted to pH 9.5, and distilled into a sulfuric acid solution. The distillate is brought up to volume, neutralized with sodium hydroxide, and analyzed by SM 4500-NH_3 G.

Source: APHA, AWWA and WEF. 2017. "Method 4500-NH3 B: Nitrogen (Ammonia) Preliminary Distillation Step." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.104 Standard Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Analyte determination and measurement
Determinative Technique: Visible spectrophotometry

Sample Preparation Method: Standard Method 4500-NH_3 B
Sample Preparation Technique: Distillation

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or ground water, and good-quality nitrified wastewater effluent
Method Selected for: This method has been selected for analysis of non-drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The range of the method in drinking water, surface, and domestic and industrial wastewaters is 0.02–2.0 mg/L.

Description of Method: Ammonia is determined as indophenol blue by this method. A portion of the neutralized sample distillate (from procedure 4500-NH_3 B [Section 5.2.103]) is run through a manifold. The ammonium in the distillate reacts with solutions of disodium ethylenediaminetetraacetic acid (EDTA), sodium phenate, sodium hypochlorite and sodium nitroprusside. The resulting indophenol blue is detected by colorimetry in a flow cell. Photometric measurement is made between the wavelengths of 630 and 660 nm.

Special Considerations: Remove interfering turbidity by filtration. Color in the samples that absorbs in the photometric range (630–660 nm) can interfere with analysis.

Source: APHA, AWWA and WEF. 2017. "Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.105 Standard Method 4500-Cl G: Chlorine (Residual) DPD Colorimetric Method

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Water samples are buffered and colorimetric agent is added. Buffered water extraction by Analyst, 1999. 124: 1853-1857 (Section 5.2.106) is used for preparation of air samples.
Determinative Technique: Visible spectrophotometry

Method Developed for: Chlorine in water and wastewater
Method Selected for: This method has been selected for preparation and analysis of water samples to address chlorine. It also has been selected for analysis of air samples when appropriate sample preparation techniques have been applied. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method can detect 10 µg/L chlorine.

Description of Method: A 10-mL portion of buffered aqueous sample is reacted with N,N-diethyl-*p*-phenylenediamine (DPD) color agent. The resulting free chlorine is determined by colorimetry. If total chlorine (including chloramines and nitrogen trichloride) is to be determined, potassium iodide crystals are added. Results for chromate and manganese are blank corrected using thioacetamide solution.

Special Considerations: Organic contaminants and strong oxidizers may cause interference. Color and turbidity in the sample can cause interference and can be compensated for by first zeroing the photometer using the sample. Chromate interferences are minimized by using thioacetamide blank correction.

Source: APHA, AWWA and WEF. 2017. "Method 4500-Cl G: DPD Colorimetric Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.106 Literature Reference for Chlorine in Air (Analyst, 1999. 124(12): 1853-1857)

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation
Sample Preparation Technique: Buffered water extraction
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-Cl G

Method Developed for: Active chlorine in air
Method Selected for: This method has been selected for preparation of air samples to address chlorine. See Appendix A for the corresponding method usability tier.

Description of Method: A procedure is described for determination of total combined gas-phase active chlorine (i.e., molecular chlorine, hypochlorous acid, and chloramines) and is based on a sulfonamide-functionalized silica gel sorbent. For determination of the collected chlorine, a modified version of the DPD colorimetric procedure is used, which yielded a detection limit of 0.1 µg of chlorine. At flow rates ranging from 31 to 294 mL/minute, the collection efficiency was >90% based on breakthrough analysis. Recovery of chlorine spikes from 0.05-g aliquots of the sorbent was not quantitative (~60%) but was reproducible; the recovery is accounted for in samples by adding weighed amounts of sorbent to the standards.

Source: Johnson, B.J., Emerson, D.W., Song, L., Floyd, J. and Tadepalli, B. 1999. "Determination of Active Chlorine in Air by Bonded Phase Sorbent Collection and Spectrophotometric Analysis." *Analyst.* 124(12): 1853-1857. http://pubs.rsc.org/en/content/articlelanding/1999/an/a906305f

5.2.107 Literature Reference for Hexamethylenetriperoxidediamine (HMTD) (Analyst, 2001. 126:1689-1693)

Analyte(s)	CAS RN
Hexamethylenetriperoxidediamine (HMTD)	283-66-9

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 8330B/3535A (solid samples and water samples), and 3570/8290A Appendix A (wipe samples). Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Determinative Technique: LC-MS-MS

Method Developed for: Trace quantities of HMTD in explosives or explosive mixtures
Method Selected for: This method has been selected for analysis of solid, water and wipe samples to address HMTD. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 20 µg/L.

Description of Method: Prepared samples are analyzed by positive mode atmospheric pressure chemical ionization (APCI) LC-MS-MS using a C_{18} analytical column (150 mm × 2.0 mm inner diameter [I.D.], 5µm particle size) coupled with a C_{18} guard cartridge system (10 mm × 2.0 mm I.D.). Elution using a 95/5 water/methanol solution detects HMTD at m/z = 209 and a retention time of ~ 15.5 minutes.

Special Considerations: The procedure has been developed for the determination of HMTD in explosives or explosive mixtures; modifications will be needed for application to environmental samples such as soils, wipes and water samples. Until modifications can be developed and tested, it is

recommended that the procedures described in SW-846 Methods 8330B and 3535A (Sections 5.2.40 and 5.2.21) be used to prepare solid and water samples, and the procedures described in SW-846 Methods 3570 and 8290A Appendix A (Sections 5.2.24 and 5.2.36) be used to prepare wipe samples.

Source: Crowson, A. and Berardah, M.S. 2001. "Development of an LC/MS Method for the Trace Analysis of Hexamethylenetriperoxidediamine (HMTD)." *Analyst*. 126(10): 1689-1693. http://pubs.rsc.org/en/Content/ArticleLanding/2001/AN/b107354k

5.2.108 Literature Reference for Cyanogen Chloride (Encyclopedia of Anal. Chem. 2006 DOI: 10.1002/9780470027318.a0809)

Analyte(s)	CAS RN
Cyanogen chloride	506-77-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Purge-and-trap, headspace, liquid-liquid microextraction
Determinative Technique: GC-MS, GC-ECD

Method Developed for: Determination of cyanogen chloride in drinking water
Method Selected for: This method has been selected for preparation and analysis of water and solid samples to address cyanogen chloride. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: In drinking water, the MDL is 0.13 µg/L when using purge-and-trap GC-MS or liquid-liquid microextraction GC-ECD, and 0.04 µg/L when using headspace GC-ECD.

Description of Method: The method describes three different sample preparation techniques (purge-and-trap, headspace and micro liquid-liquid extraction) and two different determinative techniques (GC-MS and GC-ECD). Using the purge-and-trap technique, cyanogen chloride and an internal standard are extracted (purged) from the sample matrix by bubbling an inert gas through the sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated. Simultaneously, a short piece of deactivated fused silica precolumn is cooled with liquid nitrogen to refocus the analytes. The cryotrap is heated to inject the sample onto a GC-MS.

For headspace GC-ECD analyses, a 40-mL vial is filled with sample without headspace. With the vial upside down, a volume of nitrogen is forced into the sample using a syringe, and an equivalent sample volume is dispelled through a second syringe. The sample is shaken by hand and, after settling, a volume of the headspace is sampled by syringe and injected into a split-mode GC-ECD. For liquid-liquid microextraction GC-ECD analyses, 30 mL of water sample is extracted in a 40-mL vial, with 10 g of sodium sulfate, 4 mL of MTBE and an internal standard. The sample is shaken by mechanical shaker or by hand. After allowing the phases to separate, the MTBE layer is transferred to another vial and injected into a GC-ECD.

Special Considerations: This procedure has been developed for water samples; modifications may be needed for application to environmental samples such as solid samples.

Source: Xie, Y. 2006. "Cyanogen Chloride and Cyanogen Bromide Analysis in Drinking Water." *Encyclopedia of Analytical Chemistry*. 1-11.
http://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a0809/abstract

5.2.109 Literature Reference for 3-Chloro-1,2-propanediol (Eur. J. Lipid Sci. Technol. 2011. 113: 345-355)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction, followed by SPE cleanup and derivatization
Determinative Technique: GC-MS

Method Developed for: Trace quantities of 3-chloro-1,2-propanediol in foodstuffs
Method Selected for: This method has been selected for preparation and analysis of solid and wipe samples to address 3-chloro-1,2-propanediol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The low calibration standard is 5 µg/L. The MDL in food ranges from 4 to 16 µg/kg. The working range is 4–4,000 µg/kg.

Description of Method: Foodstuffs (olive oil, cereal and potato products) are solvent extracted with hexane/diethyl ether and centrifuged. The resulting organic layer is washed several times (by adding water, vortexing and then centrifuging), then dried with sodium sulfate. The extract is concentrated to dryness, and redissolved in tetrahydrofuran (THF) to which acidified methanol is added. The reaction mixture is neutralized with sodium bicarbonate and washed with 3 aliquots of hexane, and the residue is quantitatively transferred to a sodium chloride solution. This solution is mixed with the contents of a highly pure diatomaceous earth based solid phase refill sachet, transferred to a chromatography column, and then eluted with diethyl ether. The collected eluent is concentrated by rotary evaporation and derivatized with heptafluorobutyrylimidazole (HFBI) at 70°C for 15–20 minutes. After washing with water, the extracts are analyzed using a GC-MS.

Special Considerations: The procedure has been developed for the determination of 3-chloro-1,2-propanediol in foodstuffs only; modifications may be needed for application to environmental samples.

Source: Hamlet, C. G. and Asuncion, L. 2011. "Single-Laboratory Validation of a Method to Quantify Bound 2-Chloropropane-1,3-diol and 3-Chloropropane-1,2-diol in Foodstuffs Using Acid Catalysed Transesterification, HFBI Derivatisation and GC/MS Detection." *Eur. J. Lipid Sci. Technol*. 113(3): 345-355. http://onlinelibrary.wiley.com/doi/10.1002/ejlt.v113.3/issuetoc

5.2.110 Literature Reference for Methyl Hydrazine (Journal of Chromatography B. 1993. 617(1): 157-162)

Analyte(s)	CAS RN
Methyl hydrazine	60-34-4

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: SW-846 Method 3541/3545 (for solids), SW-846 Methods 3570/8290A Appendix A (for wipes), filtration for water samples, followed by derivatization for all sample types
Determinative Technique: HPLC-UV

Method Developed for: Determination of hydrazine in human plasma
Method Selected for: This method has been selected for preparation and analysis of water samples, and for the analysis of solid and wipe samples to address methyl hydrazine. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: Detection limit in pooled plasma is 1 µg/L. The reporting range is 5–1,000 µg/L.

Description of Method: Samples are prepared in a single-step reaction by protein denaturation with trichloroacetic acid, and derivatization to a stable azine with 4-hydroxybenzaldehyde. Chromatographic separation is carried out on a reversed-phase (octadecylsilane) column with methanol:water (60:40) as the mobile phase and UV detection at 340 nm. Retention time of the azine derivative of methyl hydrazine is 3.5 minutes.

Special Considerations: This procedure has been developed for human plasma; modifications may be needed for application to environmental samples such as water, solid and wipe samples.

Source: Kircherr, H. 1993. "Determination of Hydrazine in Human Plasma by High Performance Liquid Chromatography." *Journal of Chromatography B*. 617(1): 157-162. http://www.sciencedirect.com/science/article/pii/0378434793804368

5.2.111 Literature Reference for 3-Chloro-1,2-propanediol (Journal of Chromatography A. 2000. 866(1): 65-77)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction followed by derivatization
Determinative Technique: GC-ECD

Method Developed for: Determination of 3-chloro-1,2-propanediol in water
Method Selected for: This method has been selected for preparation and analysis of water samples to address 3-chloro-1,2-propanediol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL is 0.73 µg/L. The reporting range is 11–169 µg/L.

Description of Method: Sodium sulfate, sodium bisulfate and a surrogate are added to a 5-mL sample and extracted twice with 5 mL of ethyl acetate. The two ethyl acetate extracts are combined and concentrated to 50 µL under nitrogen evaporation. Then 100 µL of acetonitrile is added, and the solution is mixed and transferred to a drying column containing sodium sulfate. An additional 100 µL of acetonitrile is used to rinse the sample vial and the rinse is transferred to the drying column. After letting the sample sit on the column for 10 minutes, it is eluted with 2 mL of acetonitrile. The dried extract is derivatized by adding 50 µL of heptafluorobutyric anhydride (HFBA) and heating at 75°C for 30 minutes. The derivatized sample is extracted with water, then hexane, followed by a saturated sodium bicarbonate solution. The aqueous layer is discarded, and the hexane layer is washed twice with sodium bicarbonate solution and shaken for 30 seconds. The hexane extract is then transferred to a GC vial and analyzed by GC-ECD with a DB5-MS column.

Special Considerations: The procedure has been tested for reagent grade water and seawater; modifications may be needed for application to environmental samples. The presence of 3-chloro-1,2-propanediol should be confirmed using either a secondary GC column or an MS.

Source: Matthew, B.M. and Anastasio, C. 2000. "Determination of Halogenated Mono-alcohols and Diols in Water by Gas Chromatography With Electron-Capture Detection." *Journal of Chromatography A*. 866(1): 65-77. http://www.sciencedirect.com/science/article/pii/S002196739901081X

5.2.112 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography A. 2007. 1139: 271-278)

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Water extraction followed by SPE cleanup and derivatization for solid and wipe samples. Use NIOSH Method S301-1 for air samples.
Determinative Technique: LC-MS

Method Developed for: Determination of fluoroacetate in food
Method Selected for: This method has been selected for preparation and analysis of solids and wipes and for the analysis of air samples to address the analytes listed in the table above as fluoroacetate ion. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 0.8 µg/L. The calibration range is 20–10,000 µg/L.

Description of Method: The method utilizes a water extraction, SPE cleanup, and LC-MS for determination of fluoroacetate as monofluoroacetate. SPE is performed using C_{18} cartridges. The LC-MS system utilizes a C_{18} column and the MS is operated in APCI negative mode. If significant interferences are observed, the method describes a qualitative procedure that can be used to confirm the presence of fluoroacetate. The sample is first prepared as described in the quantitative method. Then an aliquot is derivatized by adding 2-nitrophenylhydrazine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and pyridine buffer, and heating at 65°C for 15 minutes. The extract is then cleaned by putting it through a C_{18} cartridge. The extracts are then blown to dryness, reconstituted in 2 mL of water/methanol (20/80), and filtered through a 0.2 µm filter. Analysis of the cleaned extract is performed on an LC-MS using a C_8 column and gradient elution, beginning with 25% methanol for the first 3 minutes, followed by 80% methanol over the next 10 minutes. A post run equilibration (7 minutes) is used prior to the next injection.

Special Considerations: This procedure has been developed for food; modifications may be needed for application to environmental samples such as solid and wipe samples. In addition, the air filter extraction procedure (described in NIOSH Method S301-1) was not developed for the LC-MS-MS detector, and it may be necessary to alter the extraction method if interferences arising from the extraction are observed.

Source: Noonan, G.O., Begley, T.H. and Diachenko, G.W. 2007. "Rapid Quantitative and Qualitative Confirmatory Method for the Determination of Monofluoroacetic Acid in Foods by Liquid Chromatography–Mass Spectrometry." *Journal of Chromatography A*. 1139: 271-278. http://www.sciencedirect.com/science/article/pii/S0021967306021388

5.2.113 Literature Reference for Acephate and Methamidophos (Journal of Environmental Science and Health, Part B. 2014. 49: 23-34)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation
Sample Preparation Technique: Solvent extraction
Determinative Technique: LC-MS-MS

Determinative Method: EPA Method 538 (Section 5.2.11)
Method Developed for: Acephate and methamidophos in soil
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: 10 grams of soil is homogenized with 95 mL of an 85% sterile saline solution by shaking on a rotary shaker. Acephate and methamidophos are extracted from the soil by adding 95 mL of 0.85% saline solution to 10 g of soil sample. The mixture is agitated on a rotary shaker set at 150 rpm. The mixture is centrifuged and the aqueous layer is removed and extracted four times with an equal volume of a 1:1 diethyl ether: chloroform solution. The organic layers are combined and evaporated to dryness in a rotary evaporator. The residue is then redissolved in acetonitrile and analyzed by LC-MS-MS (see Special Considerations for notes about selecting the redissolving solvent).

Special Considerations: The procedure was developed with acetonitrile as the solvent used to redissolve the extracted residue prior to LC-MS-MS analysis using a gradient solvent system consisting of acetonitrile: water: acetic acid (40:60:0.1 v/v). Modifications to the extraction solvent may be needed when using the LC-MS-MS conditions described EPA Method 538 (Section 5.2.11), which uses a gradient solvent of 20 mM ammonium formate in reagent water. A solvent system that is appropriate for solubilizing the target compounds and is compatible with the mobile phase used in the determinative method is recommended.

Source: Ramu, S. and Seetharam, B. 2014. "Biodegradation of acephate and methamidophos by a soil bacterium *Pseudomonas aeruginosa* strain Is-6." *Journal of Environmental Science and Health, Part B*. 49: 23-34. https://www.tandfonline.com/doi/full/10.1080/03601234.2013.836868

5.2.114 Literature Reference for Acephate and Methamidophos (Journal of Chromatography A. 2007. 1154: 3-25)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: LC-MS-MS

Method Developed for: Pesticides (methamidophos) in crops
Method Selected for: This method has been selected for preparation and analysis of air and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD for this method is 0.01 mg/kg.

Description of Method: An LC-MS-MS multi-residue method for the simultaneous target analysis of a wide range of pesticides and metabolites in fruit, vegetables and cereals is described. Gradient elution has been used in conjunction with ESI+ tandem mass spectrometry to detect up to 171 pesticides and/or metabolites in different crop matrices using a single chromatographic run. Pesticide residues are extracted/partitioned from the samples with acetone/dichloromethane/light petroleum. Samples are analyzed by LC-MS-MS using a C_{18} analytical column (150 mm × 3.2 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (4 mm × 3.0 mm I.D.).

Special Considerations: The procedure has been developed for the analysis of various pesticides (methamidophos) in crops using LC-MS-MS; modifications will be needed for application to

environmental samples such as soils, wipes and air samples collected on sorbent/filters. If problems occur when using this method to analyze for methamidophos in air samples, NIOSH Method 5600 (Section 5.2.69) should be used.

Source: Hiemstra, M. and de Kok, A. 2007. "Comprehensive Multi-residue Method for the Target Analysis of Pesticides in Crops Using Liquid Chromatography-Tandem Mass Spectrometry." *Journal of Chromatography A*. 1154(1): 3-25. http://www.sciencedirect.com/science/article/pii/S0021967307005845

5.2.115 Literature Reference for Paraquat (Journal of Chromatography A. 2008, 1196-1197, 110-116)

Analyte(s)	CAS RN
Paraquat	4685-14-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extraction by digestion, shaking or microwave-assisted extraction (MAE) followed by SPE cleanup
Determinative Technique: LC-UV or LC-MS-MS

Method Developed for: Determination of quaternary ammonium herbicides in soil
Method Selected for: This method has been selected for preparation and analysis of solid and wipe samples to address paraquat. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: LODs are 10 µg/kg (digestion) and 50 µg/kg (MAE) when using LC-UV, and 1.0 µg/kg (digestion) and 3.0 µg/kg (MAE) when using LC-MS-MS. EQLs are 20 µg/kg and 100 µg/kg when using LC-UV, and 2.0 µg/kg (digestion) and 7.5µg/kg (MAE) when using LC-MS-MS.

Description of Method: Soil matrices can be extracted using one of the following three procedures: (1) digestion with an acidic methanol/ EDTA solution, (2) shaking in an EDTA/ammonium formate solution, or (3) using an MAE system in a benzalkonium chloride/acid solution. Cleanup of extracts is performed by SPE using silica cartridges for all three extraction procedures. Detection of these herbicides is carried out by either LC-UV or LC-MS-MS.

Special Considerations: This procedure has been developed for soil samples; modifications may be needed for application to environmental samples such as wipes.

Source: Pateiro-Moure, M., Martínez-Carballo, E., Arias-Estévez, M. and Simal-Gándara, J. 2008. "Determination of Quaternary Ammonium Herbicides in Soils. Comparison of Digestion, Shaking and Microwave-Assisted Extractions." *Journal of Chromatography A*. 1196-1197, 110-116. http://www.sciencedirect.com/science/article/pii/S0021967308005335

5.2.116 Literature Reference for Fentanyl (Journal of Chromatography A. 2011. 1218: 1620-1649)

Analyte(s)	CAS RN
Fentanyl	437-38-7

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 3541 and 3545A (solid samples) and 3520C and 3535A (water samples)
Determinative Technique: LC-MS-MS

Method Developed for: Fentanyl in wastewater and surface water
Method Selected for: This method has been selected for analysis of water and solid samples to address fentanyl. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs in surface water and wastewater (effluent and influent) are 0.05 and 0.2 ng/L, respectively. Method quantitation limits in surface water, wastewater effluent and wastewater influent are 0.10, 0.6 and 0.7 ng/L, respectively. The reportable range for fentanyl in surface water is 0.08–750 ng/L.

Description of Method: Water samples are vacuum filtered, first through two sequential glass fiber filters (2.7 µm, then 0.7 µm). After filtration, samples are acidified with 31% hydrochloric acid to pH 1.8–1.9. The SPE cartridge (60 mg) is conditioned with 2 mL of methanol and equilibrated with 2% formic acid in water (2 mL, pH 2), both at a flow rate of 3 mL/minute. Acidified samples are spiked with 50 ng of each surrogate and internal standard and then passed through the cartridge at a rate of 6 mL/minute. Immediately following loading, cartridges are washed with 2% formic acid in water (2 mL at pH 2) at a flow rate of 3 mL/minute, then washed again with 2 mL of 0.6% formic acid in methanol (pH 2) at a flow rate of 3 mL/minute, followed by elution with 3 mL of 7% ammonium hydroxide in methanol at a flow rate of 1 mL/minute into silanized vials. Extracts are evaporated to dryness under nitrogen, reconstituted with 500 µL of 0.3% acetic acid/5% methanol in reagent-grade water, and filtered through 0.2 µm polytetrafluoroethylene (PTFE) filters before being transferred to deactivated maximum recovery vials with PTFE septa. Extracts are analyzed by LC-MS-MS equipped with an ethylene-bridged hybrid (BEH) column.

Source: Baker, D. and Kaxprzyk-Hordern, B. 2011. "Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry." *Journal of Chromatography A*. 1218(12): 1620-1659. http://www.sciencedirect.com/science/article/pii/S0021967311001312

5.2.117 Literature Reference for BZ (Journal of Chromatography B. 2008. 874: 42–50)

Analyte(s)	CAS RN
BZ [Quinclidinyl benzylate]	6581-06-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Direct injection for water samples, SW-846 Methods 3541 or 3545 for solid samples, and SW-846 Methods 3570 and 8290A Appendix A for wipes.
Determinative Technique: LC-MS-MS

Method Developed for: Benzodiazepines in human plasma
Method Selected for: This method has been selected for preparation and analysis of water samples and for the analysis of prepared solid and wipe samples to address BZ. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The limits of detection for the benzodiazepines in human plasma ranged from 0.1 to 1 ng/mL, the LOQs ranged from 0.25 to 5 ng/mL, and the working range for the benzodiazepines in human plasma is 0.25–1000 ng/mL (depending on the analyte). This method was developed for compounds similar in structure to BZ; therefore, no detection or quantification information is available for BZ.

Description of Method: Water samples are filtered and directly injected into an LC-MS-MS for analysis. Soil samples are extracted by PFE or Soxhlet extraction using SW-846 Method 3541/3545A (Sections 5.2.22 and 5.2.23), prior to filtration and injection into the LC-MS-MS. Wipe samples are solvent extracted prior to filtration and injection into the LC-MS-MS. In all cases, an internal standard is added prior to extraction or filtration. The triple quadrupole LC-MS-MS is equipped with a C_8 column

and operated in positive ion mode with MRM. Instrument parameters specific for BZ can be found in Schaer, 2012 (see Additional Resource).

Special Considerations: The procedure has been developed for the analysis of benzodiazepines in human plasma; modifications will be needed for application to BZ in environmental samples. An overview of a strategy for detection and identification of BZ, including information regarding ESI-source, fragmentation, precursor-ion, collision energies, and scanning time, is provided in a poster presentation in the Additional Resource cited below.

Source: Abbara, C., Bardot, I., Cailleux, A., Lallement, G., Le Bouil, A., Turcant, A., Clair, P. and Diquet, B. 2008. "High-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of diazepam, atropine and pralidoxime in human plasma." *Journal of Chromatography B*. 874: 42-50. http://www.sciencedirect.com/science/article/pii/S1570023208006545

Additional Resource: Schaer, M. Poster Presentation "Rapid Screening and Identification of Chemical Warfare Agents in Environmental Samples using LC/MS and a MS/MS-Library." Spiez Laboratory, CH-3700, Spiez, Switzerland. https://www.spiezlab.admin.ch/en/ls/ueberuns.html. Contact: laborspiez@babs.admin.ch.

5.2.118 Literature Reference for Fluoroacetamide (Journal of Chromatography B. 2008. 876(1): 103-108)

Analyte(s)	CAS RN
Fluoroacetamide	640-19-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction
Determinative Technique: GC-MS

Method Developed for: Fluoroacetamide and tetramine in blood, urine and stomach contents
Method Selected for: This method has been selected for preparation and analysis of solid, water, air and wipe samples to address fluoroacetamide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit of this method for fluoroacetamide is 0.01 µg/g.

Description of Method: Samples are extracted by microscale liquid-liquid extraction using acetonitrile, ENVI-Carb™ (Sigma-Aldrich, St. Louis, MO) and sodium chloride. Samples are analyzed by GC-MS using a 30-m DB-5MS capillary column (or equivalent) coupled with a 1.5 m Innowax capillary column (or equivalent) by a quartz capillary column connector. If analyzing for fluoroacetamide alone, only the Innowax capillary column is needed.

Special Considerations: The procedure has been developed for the analysis of fluoroacetamide and tetramine in blood, urine and stomach fluid samples; modifications will be needed for application to environmental samples.

Source: Xu, X., Song, G., Zhu, Y., Zhang, J., Zhao, Y., Shen, H., Cai, Z., Han, J. and Ren, Y. 2008. "Simultaneous Determination of Two Acute Poisoning Rodenticides Tetramine and Fluoroacetamide With a Coupled Column in Poisoning Cases." *Journal of Chromatography B*. 876(1): 103-108. http://www.sciencedirect.com/science/article/pii/S1570023208007757

5.2.119 Literature Reference for Carfentanil and 3-Methyl Fentanyl (J. Chromatogr. B. 2014. 962: 52-58)

Analyte(s)	CAS RN
Carfentanil	59708-52-0
3-Methyl fentanyl	42045-87-4

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 3541 and 3545A (solid samples) and 3520C and 3535A (water samples)
Determinative Technique: LC-MS-MS

Method Developed for: Carfentanil, fentanyl and 3-methyl fentanyl in human urine
Method Selected for: This method has been selected for analysis of water, and solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: LODs for carfentanil, fentanyl and 3-methyl fentanyl in human urine when using off-line SPE are estimated to be 0.008, 0.007 and 0.020 ng/mL, respectively. The reportable range is 0.010–10 ng/mL for carfentanil and fentanyl, and 0.050–10 ng/mL for 3-methyl fentanyl.

Description of Method: Carfentanil, fentanyl and 3-methyl fentanyl are extracted from samples by placing aliquots off-line on a 96-well plate or using an on-line SPE system. In both cases, internal standards are added to sample aliquots prior to loading onto the SPE cartridge. For on-line SPE, cartridges are conditioned with acetonitrile and aqueous ammonium hydroxide solutions. Sample aliquots are loaded onto the SPE unit, washed with aqueous ammonium hydroxide:acetonitrile solution and eluted with LC gradient onto the HPLC column. When using the 96-well plate, sample aliquots are diluted with aqueous ammonium hydroxide and loaded onto a plate conditioned with acetonitrile and aqueous ammonium hydroxide solutions, washed with a water:acetonitrile:ammonium hydroxide mixture, and eluted with acetonitrile. The extracts are evaporated to dryness, reconstituted in water and injected into the HPLC for analysis. Target compounds are identified using ESI tandem mass spectrometry. The retention time for all three compounds is expected to be ~3.4 minutes. The analytes can be measured as individual compounds due to the use of different monitoring ions.

Special Considerations: This procedure has been developed for urine samples; modifications may be needed for application to environmental samples.

Source: Shaner, R. L., Kaplan, P., Hamelin, E. I., William A. Bragg, W. A., Johnson, R. C. 2014. "Comparison of two automated solid phase extractions for the detection of ten fentanyl analogs and metabolites in human urine using liquid chromatography tandem mass spectrometry." *Journal of Chromatography B*. 962: 52-55.
http://www.sciencedirect.com/science/article/pii/S157002321400333X?via%3Dihub

5.2.120 Literature Reference for Sodium Azide (Journal of Forensic Sciences. 1998. 43(1): 200-202)

Analyte(s)	CAS RN
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction, filtration and/or acidification
Determinative Technique: IC with conductivity detection
Determinative Method: EPA Method 300.1, Revision 1.0

Method Developed for: Sodium azide in blood
Method Selected for: This method has been selected for preparation of solid and water samples to address sodium azide as azide ion. See Appendix A for corresponding method usability tiers.

Description of Method: Samples are analyzed by IC using suppressed conductivity detection. Water extraction and filtration steps should be used for the preparation of solid samples. Filtration steps should be used for preparation of aqueous liquid and drinking water samples.

Special Considerations: The procedure was developed for analysis of sodium azide in blood samples; modifications may be needed for application to environmental samples.

Source: Kruszyna, R., Smith, R.P. and Kruszyna, H. 1998. "Determining Sodium Azide Concentration in the Blood by Ion Chromatography." J*ournal of Forensic Sciences*. 43(1): 200-202.
http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS16113J.htm

Section 6.0: Selected Radiochemical Methods

A list of analytical methods to be used in analyzing environmental and outdoor building and infrastructure material samples for radiochemical contaminants following a contamination incident is provided in Appendix B. Methods are listed for each isotope and for each sample type that potentially may need to be measured and analyzed when responding to a radiological or nuclear incident. The isotopes included are based on selection criteria that address the needs and priorities of EPA as well as other federal agencies (see Section 1.0).

Please note: This section provides guidance for selecting radiochemical methods to facilitate data comparability when laboratories are tasked with analyzing samples following a large scale radiological or nuclear contamination incident. Although the majority of methods have been validated for the analyte/sample type combination for which they have been selected, validation is still needed for a few of the methods that have been selected for analysis of vegetation. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix B1 (environmental samples) is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The radionuclide(s) or contaminant(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names. In this section (Section 6.0) and Appendix B, the CAS RNs correspond to the specific radionuclide identified.
- **Determinative technique.** An analytical instrument or technique used for qualitative and confirmatory determination of compounds or components in a sample.
- **Drinking water sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in drinking water samples. Methods have been identified for qualitative and confirmatory determination.
- **Aqueous- and liquid-phase sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in aqueous- and/or non-aqueous liquid-phase (aqueous/liquid-phase) samples. Methods have been identified for qualitative and confirmatory determination.
- **Soil and sediment sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in soil and sediment samples. Methods have been identified for qualitative and confirmatory determination.
- **Surface wipe sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in surface wipe samples. Methods have been identified for qualitative and confirmatory determination.
- **Air filter sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in air filter samples. Methods have been identified for qualitative and confirmatory determination.
- **Vegetation sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in vegetation (i.e., grasses, leaves, trees, etc.) not intended for human consumption. Methods have been identified for qualitative and confirmatory determination.

- **Qualitative determination method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is intended to determine the presence of a radionuclide. Although quantitative, these methods have been selected as qualitative methods since they can be utilized with shorter counting times, at greater uncertainty, when increased sample throughput and more rapid reporting of results are required.
- **Confirmatory method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is for measurement of the activity from a particular radionuclide per unit of mass, volume or area sampled.

Appendix B2 (outdoor building and infrastructure materials) is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The radionuclide(s) or contaminant(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names. In this section (Section 6.0) and Appendix B, the CAS RNs correspond to the specific radionuclide identified.
- **Determinative technique.** An analytical instrument or technique used for qualitative and confirmatory determination of compounds or components in a sample.
- **Asphalt shingle sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in asphalt roofing materials. Methods have been identified for sample preparation and confirmatory determination.
- **Asphalt matrices sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in asphalt paving material samples. Methods have been identified for sample preparation and confirmatory determination.
- **Concrete sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in concrete samples. Methods have been identified for sample preparation and confirmatory determination.
- **Brick sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in brick samples. Methods have been identified for sample preparation and confirmatory determination.
- **Limestone sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in limestone samples. Methods have been identified for sample preparation and confirmatory determination.
- **Sample preparation method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is intended to digest solid samples into a liquid form suitable for analysis.
- **Confirmatory method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is for measurement of the activity from a particular radionuclide per unit of mass, volume or area sampled.

Following a contamination incident, it is assumed that only those areas with contamination greater than pre-existing/naturally prevalent levels (i.e., background) commonly found in the environment or on buildings and infrastructure would be subject to remediation. Dependent on site- and event-specific goals, investigation of background levels using methods listed in Appendix B is recommended.

In some cases, the availability of reagents and standards required for the selected analytical methods might be limited. In these cases, the radiochemistry methods points of contact listed in Section 4.0 should be contacted for additional information.

6.1 General Guidelines

The guidelines summarized in this section provide a general overview of how to identify the appropriate radiochemical method(s) for a given analyte-sample type combination, as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the radionuclides listed in Appendix B, EPA's Radiation Protection Program (https://www.epa.gov/radiation/radionuclides) and the *Multi-Agency Radiological Laboratory Analytical Protocols Manual* (MARLAP) (https://www.epa.gov/radiation/marlap-manual-and-supporting-documents) websites provide information pertaining to radionuclides of interest and selection of radiochemical methods. Documents for emergency response operations for laboratories, developed by EPA's Office of Radiation and Indoor Air (ORIA), describe the likely analytical decision paths that would be required by personnel at a radioanalytical laboratory following a radiological or nuclear contamination incident. These documents may be found at https://www.epa.gov/radiation/radiation-protection-document-library (enter "incident guides" in the search bar for quick access).

6.1.1 Standard Operating Procedures for Identifying Radiochemical Methods

To determine the appropriate method to be used on an environmental sample, locate the analyte of concern in Appendix B1: Selected Radiochemical Methods under the "Analyte Class" or "Analyte(s)" column. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., alpha spectrometry), then identify the appropriate qualitative and/or confirmatory method for the sample type of interest (drinking water, aqueous/liquid-phase, soil and sediment, surface wipes, air filters and vegetation) for the particular analyte.

To determine the appropriate method to be used on an outdoor building or infrastructure material sample, locate the analyte of concern in Appendix B2: Selected Radiochemical Methods under the "Analyte Class" or "Analyte(s)" column. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., alpha spectrometry), then identify the appropriate sample preparation and/or confirmatory method for the sample type of interest (asphalt shingles, asphalt materials, concrete, brick or limestone).

Once a method has been identified in Appendix B1 or B2, **Table 6-1** can be used to locate the method summary. Sections 6.2.1 through 6.2.61 provide summaries of the qualitative and confirmatory methods listed in Appendix B1 for analysis of environmental samples. Sections 6.3.1 through 6.3.9 provide summaries of the sample preparation and confirmatory methods listed in Appendix B2 for analysis of outdoor building and infrastructure material samples.

Table 6-1. Radiochemical Methods and Corresponding Section Numbers

Analyte / Analyte Class	CAS RN	Method	Section
Gross Alpha Gross Beta	NA NA	900.0 (EPA)	6.2.2
		FRMAC, Vol 2, pg. 33 (DOE)	6.2.38
		AP1 (ORISE)	6.2.42
		7110 B (SM)	6.2.52
Gamma Select Mixed Fission Products	NA	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Total Activity Screening	NA	Y-12 Preparation of Samples for Total Activity Screening (DOE)	6.2.58
Actinium-225	14265-85-1	900.0 (EPA)	6.2.2
		FRMAC, Vol 2, pg. 33 (DOE)	6.2.38
		AP1 (ORISE)	6.2.42
		7110 B (SM)	6.2.52
		Determination of ^{225}Ac in Water Samples (Eichrom)	6.2.60
		Determination of ^{225}Ac in Geological Samples (Eichrom)	6.2.61
Americium-241	14596-10-2	***Alpha Spectrometry:***	
		Rapid Radiochemical Method for Am-241 (EPA)	6.2.11
		Rapid methods for acid or fusion digestion (EPA)	6.2.16 and 6.2.17
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.19
		Am-01-RC (HASL-300)	6.2.27
		Am-04-RC (HASL-300)	6.2.28
		Am-06-RC (HASL-300)	6.2.29
		Pu-12-RC (HASL-300)	6.2.32
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.41
		AP11 (ORISE)	6.2.46
		D3084-20 (ASTM)	6.2.48
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Americium-241 in Building Materials (EPA)	6.3.6
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	6.3.9
		Gamma Spectrometry:	
		901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
		7120 (SM)	6.2.53

Analyte / Analyte Class	CAS RN	Method	Section
Californium-252	13981-17-4	Rapid Radiochemical Method for Californium-252 (EPA)	6.2.22
		Am-06-RC (HASL-300)	6.2.29
		AP11 (ORISE)	6.2.46
		D3084-20 (ASTM)	6.2.48
Cesium-137	10045-97-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Cobalt-60	10198-40-0	7120 (SM)	6.2.53
Curium-244	13981-15-2	Rapid Radiochemical Method for Curium-244 in Water (EPA)	6.2.23
		Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	6.2.24
		Am-06-RC (HASL-300)	6.2.29
		AP11 (ORISE)	6.2.46
		D3084-20 (ASTM)	6.2.48
Europium-154	15585-10-1	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
		7120 (SM)	6.2.53
Gallium-68	15757-14-9	901.1 (EPA)	6.2.3
Germanium-68	15756-77-1	Ga-01-R (HASL-300)	6.2.30
Indium-111	15750-15-9	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Iodine-125	14158-31-7	Procedure #9 (ORISE)	6.2.47
Iodine-131	10043-66-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Iridium-192	14694-69-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
		7120 (SM)	6.2.53
Molybdenum-99	14119-15-4	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Neptunium-237	13994-20-2	907.0 (EPA)	6.2.6
		SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.26
Neptunium-239	13968-59-7	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
		7120 (SM)	6.2.53
Phosphorus-32	14596-37-3	Rapid Radiochemical Method for P-32 (EPA)	6.2.8
		R4-73-014 (EPA)	6.2.9
		RESL P-2 (DOE)	6.2.39

Analyte / Analyte Class	CAS RN	Method	Section
Plutonium-238 Plutonium-239	13981-16-3 15117-48-3	EMSL-33 (EPA)	6.2.7
		Rapid Radiochemical Method for Pu-238 and -239/240 (EPA)	6.2.12
		Rapid methods for acid or fusion digestion (EPA)	6.2.16 and 6.2.17
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.19
		Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices (EPA)	6.2.21
		SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.26
		Am-06-RC (HASL-300)	6.2.29
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.41
		AP11 (ORISE)	6.2.46
		D3084-20 (ASTM)	6.2.48
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)	6.3.5
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	6.3.9
Polonium-210	13981-52-7	Method 111 (EPA)	6.2.1
		Po-02-RC (HASL-300)	6.2.31
Radium-223	15623-45-7	Rapid Radiochemical Method for Ra-226 in Water (EPA)	6.2.13
Radium-226	13982-63-3	Rapid Radiochemical Method for Ra-226 in Water (EPA)	6.2.13
		Rapid methods for acid or fusion digestion (EPA)	6.2.16 and 6.2.17
		Rapid Method for Radium in Soil (EPA)	6.2.18
		Ra-03-RC (HASL-300)	6.2.33
		AP7 (ORISE)	6.2.45
		7500-Ra B (SM)	6.2.54
		7500-Ra C (SM)	6.2.55
		Method for Radium-228 and Radium-226 in Drinking Water by Gamma-ray Spectrometry (GA Tech)	6.2.59
		Rapid Radiochemical Method for Radium-226 in Building Materials (EPA)	6.2.25 and 6.3.2
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	6.3.9

Analyte / Analyte Class	CAS RN	Method	Section
Rhenium-188	14378-26-8	901.1 (EPA)	6.2.3
Rubidium-82	14391-63-0	Ga-01-R (HASL-300)	6.2.30
Ruthenium-103	13968-53-1	901.1 (EPA)	6.2.3
Ruthenium-106	13967-48-1	Ga-01-R (HASL-300)	6.2.30
Selenium-75	14265-71-5	7120 (SM)	6.2.53
Strontium-89	14158-27-1	905.0 (EPA)	6.2.4
		Strontium in Food and Bioenvironmental Samples (EPA)	6.2.10
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2.40
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.41
Strontium-90	10098-97-2	905.0 (EPA)	6.2.4
		Rapid Radiochemical Method for Radiostrontium (EPA)	6.2.14
		Rapid methods for acid or fusion digestion (EPA)	6.2.16 and 6.2.17
		Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses (EPA)	6.2.20
		Sr-03-RC (HASL-300)	6.2.34
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.41
		D5811-20 (ASTM)	6.2.50
		Rapid Method for Total Radiostrontium in Building Materials (EPA)	6.3.1
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	6.3.9
Technetium-99	14133-76-7	Tc-01-RC (HASL-300)	6.2.35
		Tc-02-RC (HASL-300)	6.2.36
		AP5 (ORISE)	6.2.44
		D7168-16 (ASTM)	6.2.51
Technetium-99m	378784-45-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.30
Thorium-227	15623-47-9	907.0 (EPA)	6.2.6
Thorium-228	14274-82-9		
Thorium-230	14269-63-7	SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.26
Thorium-232	7440-29-1		
Tritium (Hydrogen-3)	10028-17-8	906.0 (EPA)	6.2.5
		AP2 (ORISE)	6.2.43

Analyte / Analyte Class	CAS RN	Method	Section
		Rapid Radiochemical Method for Isotopic Uranium in Water (EPA)	6.2.15
		Rapid methods for acid or fusion digestion (EPA)	6.2.16 and 6.2.17
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.19
		U-02-RC (HASL-300)	6.2.37
		Actinides and Sr-89/90 in Vegetation(SRS)	6.2.41
		AP11 (ORISE)	6.2.46
		D3972-09 (2015) (ASTM)	6.2.49
Uranium-234	13966-29-5	7500-U B (SM)	6.2.56
		7500-U C (SM)	6.2.57
Uranium-235	15117-96-1	SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.26
Uranium-238	7440-61-1	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Isotopic Uranium in Building Materials (EPA)	6.3.4
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	6.3.9

The method summaries are listed in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), academia and vendors. Methods are listed in numerical order under each publisher. Where available, a direct link to the full text of the selected analytical method is provided in the method summary. For additional information regarding sample preparation and analysis procedures and on methods available through consensus standards organizations, please use the contact information provided in **Table 6-2**.

Table 6-2. Sources of Radiochemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
Code of Federal Regulations (CFR) Promulgated Test Methods	EPA, Emission Measurement Center (EMC)	https://www.epa.gov/emc/emc-promulgated-test-methods
Prescribed Procedures for Measurement of Radioactivity in Drinking Water (EPA-600 4-80-032, August 1980)	EPA, ORD, Environmental Monitoring and Support Laboratory (EMSL)	https://nepis.epa.gov/Exe/ZyPDF.cgi/30000QHM.PDF?Dockey=30000QHM.PDF Also available from National Technical Information Service (NTIS)*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.

Name	Publisher	Reference
Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events	EPA, ORIA, National Analytical and Radiation Environmental Laboratory (NAREL)	https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides
Rapid Radiochemical Methods for Selected Radionuclides	EPA, ORIA, NAREL	https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides
Radiochemical Analytical Procedures for Analysis of Environmental Samples, March 1979. EMSL-LV-0539-17	EPA, EMSL	Available NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
EML Procedures Manual, Health and Safety Laboratory (HASL-300), 28th Edition, February, 1997	Department of Energy (DOE), Environmental Measurements Laboratory (EML) / Now DHS	http://www.wipp.energy.gov/NAMP/EMLLegacy/ Also available from NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Federal Radiological Monitoring and Assessment Center (FRMAC) Laboratory Manual	DOE, National Nuclear Security Administration (NNSA)	https://www.epa.gov/sites/production/files/2015-06/documents/frmac-vol2-pg33.pdf
Y-12 National Security Complex (Y-12)	DOE, NNSA	http://www.y12.doe.gov/
Radiological and Environmental Sciences Laboratory (RESL) Analytical Chemistry Branch Procedures Manual	DOE, RESL	Available from NTIS, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Savannah River Site (SRS) Methods	DOE, SRS	Savannah River National Laboratory Savannah River Site Aiken, SC 29808, (803) 725-6211.
Annual Book of ASTM Standards, Vol. 11.02*	ASTM International	http://www.astm.org
Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2017*	American Public Health Association (APHA)	http://www.standardmethods.org
Method for the Determination of Radium-228 and Radium-226 in Drinking Water by Gamma-ray Spectrometry Using HPGE or Ge(Li) Detectors, Georgia Institute of Technology, Environmental Resource Center	Georgia Institute of Technology, Environmental Resource Center	https://www.regulations.gov/document/EPA-HQ-OW-2018-0558-0048
Eichrom Technologies, LLC Application Notes	Eichrom Technologies, LLC	https://www.eichrom.com/eichrom/applications-notes/

* Subscription and/or purchase required.

6.1.2 General QC Guidelines for Radiochemical Methods

Having data of known and documented quality is critical so that public officials can accurately assess the activities that may be needed in remediating a site and determine the effectiveness of those activities.[10] Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated. Various levels of QC may be required if the data are generated during contaminant presence/absence qualitative determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and data quality objectives (DQOs) should be derived based on those needs and should be applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening analyses, minimal QC samples (e.g., blanks, duplicates) and documentation might be used. Implementation of the analytical methods for evaluation of environmental and outdoor building and infrastructure material samples during site assessment through site clearance, such as those identified in this document, might require increased QC frequency and more stringent QC criteria.

Some method-specific QC requirements are described in many of the individual methods that are cited in this manual. QC requirements will be referenced in analytical protocols developed to address specific analytes and sample types of concern. Additional information regarding QC requirements specific to radiochemical methods is included in the MARLAP manual at: https://www.epa.gov/radiation/radiation-protection-document-library (enter "MARLAP" in the search for quicker access). Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine any additional QC that may be needed.

QC samples are required to assess the precision, bias and reliability of sample results. All QC results are tracked on control charts and reviewed for acceptability and trends in analysis or instrument operation. QC parameters are measured as required per method at the prescribed frequency. QC of laboratory analyses using radiochemical methods includes ongoing analysis of QC samples and tracking QC parameters including, but not limited to the following:

- Method blanks
- Calibration checks
- Sample and sample duplicates
- Laboratory control sample recoveries
- Matrix spike/matrix spike duplicate (MS/MSD) recoveries and precision
- Tracer and/or carrier yield

Please note: The type and quantity of appropriate quality assurance (QA) and QC procedures that will be required are incident-specific and should be included in incident-specific documents (e.g., Quality Assurance Project Plan [QAPP], Sampling and Analysis Plan [SAP], laboratory Statement of Work [SOW], analytical methods). This documentation and/or Incident Command should be consulted regarding appropriate QA and QC procedures prior to sample analysis.

[10] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality/guidance-systematic-planning-using-data-quality-objectives-process-epa-qag-4.

6.1.3 Safety and Waste Management

It is imperative that safety precautions be used during collection, processing and analysis of environmental and outdoor building and infrastructure material samples. Laboratories should have a documented radiation safety plan or manual in addition to a health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures in the plan or manual. In addition, many of the methods summarized or cited in Section 6.2 and Section 6.3 contain specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing samples and reagents. These methods may also provide information regarding waste management. Laboratories should consult with the responsible government agencies prior to disposal of waste materials. Other resources that can be consulted for additional information include the following:

- Occupational Safety and Health Administration (OSHA) - 29 CFR part 1910.1450. *Occupational Exposure to Hazardous Chemicals in Laboratories*. Available at: https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.1450
- EPA - 40 CFR part 260. *Hazardous Waste Management System: General*. Available at: https://www.gpo.gov/fdsys/granule/CFR-2012-title40-vol27/CFR-2012-title40-vol27-part260
- EPA - 40 CFR part 270. *EPA Administered Permit Programs: The Hazardous Waste Permit Program*. Available at: https://www.gpo.gov/fdsys/granule/CFR-2012-title40-vol28/CFR-2012-title40-vol28-part270
- U.S. Nuclear Regulatory Commission (NRC) - 10 CFR part 20. *Standards for Protection Against Radiation*. Available at: https://www.ecfr.gov/current/title-10/chapter-I/part-20?toc=1
- DOE. *Order O 435.1: Radioactive Waste Management*. January 1, 2007. Available at: https://www.directives.doe.gov/directives-documents/400-series/0435.1-BOrder-chg1
- DOE. M 435.1-1. *Radioactive Waste Management Manual*. Office of Environmental Management. June 8, 2011. Available at: https://www.directives.doe.gov/directives-documents/400-series/0435.1-DManual-1-chg2-AdmChg
- DOE. *Compendium of EPA-Approved Analytical Methods for Measuring Radionuclides in Drinking Water*. Prepared by the Office of Environmental Policy and Assistance Air, Water and Radiation Division (EH-412). June 1998. Available at: https://www.epa.gov/sites/default/files/2019-06/documents/compendium_of_epa-approved_analytical_methods_for_measuring_radionuclides_in_drinking_water.pdf
- EPA. 1996. *Profile and Management Options for EPA Laboratory Generated Mixed Waste*. ORIA, Washington, DC. EPA 402-R-96-015. Available at: https://www.epa.gov/sites/default/files/2015-05/documents/402-r-96-015.pdf
- EPA. 2001. *Changes to 40 CFR 266 (Storage, Treatment, Transportation, and Disposal of Mixed Waste)*. Federal Register 66:27217-27266, May 16, 2001. Available at: https://www.federalregister.gov/documents/2001/05/16/01-11411/hazardous-waste-identification-rule-hwir-revisions-to-the-mixture-and-derived-from-rules
- EPA. 2014. *Resource Conservation and Recovery Act* (*RCRA) Orientation Manual.* Office Of Resource Conservation And Recovery (ORCR), Washington, DC. EPA530-F-11-003. 242 pp. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/rom.pdf
- MARLAP Manual. 2004. Chapter 17. *Waste Management in a Radioanalytical Laboratory*. EPA 402-B-04-001B. Available at: https://www.epa.gov/sites/production/files/2015-05/documents/402-b-04-001b-17-final.pdf
- National Research Council. 1995. *Prudent Practices in the Laboratory; Handling and Disposal of Chemicals.* National Academy Press, Washington, DC. Available at: http://books.nap.edu/openbook.php?isbn=0309052297

- National Council on Radiation Protection and Measurements (NCRP). 2002. *Risk-Based Classification of Radioactive and Hazardous Chemical Wastes*, Report Number 139. 7910 Woodmont Avenue, Suite 400, Bethesda, MD 20814–3095.
- NRC / EPA. 1995. *Joint Nuclear Regulatory Commission/Environmental Protection Agency Guidance on the Storage of Mixed Radioactive and Hazardous Waste*. Federal Register 60:40204-40211.

6.2 Method Summaries (Environmental Samples)

Summaries corresponding to the methods selected for analysis of environmental samples listed in Appendix B1 are provided in Sections 6.2.1 through 6.2.61. These summaries contain information that has been extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix B1 to which the method applies, a brief description of the analytical method, and a link to the full version of the method or a source for obtaining a full version of the method. Summaries are provided for informational use. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

6.2.1 EPA Method 111: Determination of Polonium-210 Emissions from Stationary Sources

Analyte(s)	CAS RN
Polonium-210	13981-52-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Polonium-210 in particulate matter samples collected from stationary source exhaust stacks
Method Selected for: This method has been selected for qualitative and confirmatory analysis of surface wipes and air filters.

Description of Method: This method covers the determination of polonium-210 in particulate matter samples collected from stationary sources such as exhaust stacks. Polonium-210 in the sample is put in solution, deposited on a metal disc, and the radioactive disintegration rate measured. Polonium in acid solution spontaneously deposits onto the surface of metals that are more electropositive than polonium. Polonium-209 tracers should be added to each sample to determine the chemical yield.

Special Considerations: Compounds, such as clays that are present in some decontamination agents, can contain iron, magnesium and/or calcium, which can potentially be released as ions via ion exchange in the presence of certain radionuclides, and cause analytical interferences. Although iron (III), a major interference in the analysis of polonium-210 by alpha spectrometry, is extracted from the concentrated hydrochloric solution using liquid-liquid extraction with diisopropyl ether, high concentrations of iron may not be completely removed. Chelators, also present in some decontamination agents, can tightly complex iron that may be present in the sample, preventing its removal.

Source: U.S. EPA. EMC, prepared by the Office of Air Quality Planning and Standards (OAQPS). 2000. "Method 111: Determination of Polonium-210 Emissions from Stationary Sources." Research Triangle Park, NC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-111.pdf

6.2.2 EPA Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta particle activities in drinking water.
Method Selected for: This method has been selected for gross alpha and gross beta determination in drinking water samples and qualitative analysis of actinium-225 in drinking water samples.

Description of Method: The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Actinium-225 (CAS RN 14265-85-1) Alpha emitter
- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Thorium-227 (CAS RN 15623-47-9) Alpha emitter
- Thorium-228 (CAS RN 14274-82-9) Alpha emitter
- Thorium-230 (CAS RN 14269-63-7) Alpha emitter
- Thorium-232 (CAS RN 17440-29-1) Alpha emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

An aliquot of a preserved drinking water sample is evaporated to a small volume (3 to 5 mL) and transferred quantitatively to a tared 2-inch planchet. The aliquot volume is determined based on a maximum total solids content of 100 mg. The sample aliquot is evaporated to dryness in the planchet to a constant weight, cooled, and counted using a gas proportional or scintillation counting system. The counting system is calibrated with thorium-230 for gross alpha, and with strontium-90 for gross beta analysis.[11] A traceable standards-based efficiency curve must be developed for each calibration nuclide (thorium-230 and strontium-90) based on a range of total solids content in the 2-inch planchet from 0 to 100 mg (see method for specific recommendations and requirements for the use of cesium-137).

Special Considerations: Long counting time and increased sample size may be required to meet detection limits. Sensitivity is limited by the concentration of solids in the sample. The method provides an overall measure of alpha and beta activity, including activity for the radionuclides listed above, but does not permit the specific identification of any alpha or beta emitting radionuclides. Compounds containing carbonate, fluoride, hydroxide, or phosphate, such as those present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of radionuclides in cases where an aliquot of a water sample is transferred and analyzed separately from the entire sample.

Gross alpha screening may be used for qualitative analysis of actinium-225. For every one actinium-225 decay, there are up to four alpha particles emitted depending on daughter equilibrium. To determine the qualitative result for actinium-225, the gross alpha result should be divided by four.

[11] EPA lists standards for use when analyzing drinking water in the table at 40 CFR 141.25 (Footnote 11).

Source: U.S. EPA, EMSL. 2018. "Method 900.0, Revision 1.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA 815-B-18-002.
https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100U1ZJ.txt

6.2.3 EPA Method 901.1: Gamma Emitting Radionuclides in Drinking Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Gallium-68	15757-14-9
Gamma	NA
Germanium-68	15756-77-1
Iodine-131	10043-66-0
Indium-111	15750-15-9
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Neptunium-239	13968-59-7
Rhenium-188	14378-26-8
Rubidium-82	14391-63-0
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA
Technetium-99m	378784-45-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of select gamma emitters in drinking water samples.

Description of Method: This method is applicable for analysis of water samples that contain radionuclides that emit gamma photons with energies ranging from approximately 60 to 2000 keV. The method uses gamma spectroscopy for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. A homogeneous aliquot of water is placed into a standard geometry (normally a Marinelli beaker) for gamma counting, typically using a high purity germanium detector. Detectors such as germanium (lithium) or thallium-activated sodium iodide also can be used. Sample aliquots are counted long enough to meet the required sensitivity of measurement. To reduce adsorbance of radionuclides on the walls of the counting container, the sample is acidified at collection time. Due to its poorer resolution, significant interference can occur using the thallium-activated sodium iodide detector when counting a sample containing radionuclides that emit gamma photons of similar energies. When using this method, shielding is needed to reduce background interference. Detection limits are, in general, dependent on analyte radionuclide gamma-ray abundance, sample volume, geometry (physical shape) and counting time.

Special Considerations: The presence of reducing agents, such as those contained in some decontamination agents, can convert radionuclides to an insoluble zero-valent state that can precipitate

out of solution. Although the addition of nitric acid can prevent this precipitation from occurring, iridium, molybdenum and ruthenium would likely still precipitate in the presence of these agents. Compounds such as clays, which are also present in some decontamination agents, can sequester cesium-137, which would only be released upon complete dissolution when using this method. Compounds containing carbonate, fluoride, hydroxide or phosphate also can precipitate radionuclides out of solution. All of these are a concern in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

For qualitative analysis of the germanium-68 and gallium-68 pair, long count times may be required to meet detection limits as the 1077 KeV peak has a 3% abundance; for confirmatory analysis, the 511 KeV (176% abundance) should be larger than normal.

When detecting rubidium-82 (75 second half-life) by gamma spectroscopy in environmental samples, it is measured in equilibrium with its parent, strontium-82 (25.5 day half-life).

Source: U.S. EPA, EMSL. 1980. "Method 901.1: Gamma Emitting Radionuclides in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-901.1.pdf

6.2.4 EPA Method 905.0: Radioactive Strontium in Drinking Water

Analyte(s)	CAS RN
Strontium-89	14158-27-1
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-89, strontium-90 and total strontium in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid-phase and drinking water samples for strontium-89 and confirmatory analysis of drinking water samples for strontium-90.

Description of Method: Stable strontium carrier is added to the water sample. Both strontium-89 and strontium-90 are precipitated from the solution as insoluble carbonates. Interferences from calcium and from some radionuclides are removed by one or more precipitations of the strontium carrier as strontium nitrate. Barium and radium are removed by precipitation as chromates. The yttrium-90 decay product of strontium-90 is removed by a hydroxide precipitation step. The separated strontium-89 and strontium-90 are precipitated as carbonates, weighed for determination of the chemical recovery, and counted for beta particle activity. The counting result, ascertained immediately after separation, represents the total strontium activity (strontium-89 and strontium-90) plus an insignificant fraction of the yttrium-90 that has grown into the separated strontium-90. The yttrium-90 decay product is allowed to in-grow for approximately two weeks and then is separated with stable yttrium carrier as hydroxide and finally precipitated as the oxalate, weighed for chemical recovery, and mounted for beta counting. The strontium-90 concentration is determined from the yttrium-90 activity; strontium-89 concentration is determined from the difference.

Special Considerations: Certain chelating compounds found in decontamination agents can tightly complex barium, iron, lead, magnesium and potassium, causing interference when analyzing for strontium-89 and -90. Compounds containing carbonate, fluoride, phosphate or sulfate, which are also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of strontium in cases where an aliquot of water sample is

transferred and analyzed separately from the entire sample.

Source: U.S. EPA, EMSL. 1980. "Method 905.0: Radioactive Strontium in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-905.0.pdf

6.2.5 EPA Method 906.0: Tritium in Drinking Water

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium (as T_2O or HTO) in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water and aqueous/liquid-phase samples.

Description of Method: An unpreserved 100-mL aliquot of a drinking water sample is distilled after adjusting pH with a small amount of sodium hydroxide and adding potassium permanganate. The alkaline treatment prevents other radionuclides, such as radioiodine and radiocarbon, from distilling with the tritium. The permanganate treatment oxidizes trace organics that may be present in the sample and prevents their appearance in the distillate. To determine the concentration of tritium, the middle fraction of the distillate is used, because the early and late fractions are more apt to contain materials interfering with the liquid scintillation counting process. A portion of this collected fraction is added to a liquid scintillator cocktail, and the solution is mixed, dark adapted and counted for beta particle activity. The efficiency of the system can be determined by the use of prepared tritiated water (HTO) standards having the same density and color as the sample.

Special Considerations: Some compounds present in decontamination agents, such as organic compounds containing oxygen, reductants, halogenated compounds or elevated levels of nitrates or nitromethane, can cause chemical quenching. Color quenching compounds, such as dyes and pigments also contained in some decontamination agents, can have a significant impact when using liquid scintillation methods.

Source: U.S. EPA, EMSL. 1980. "Method 906.0: Tritium in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-906.0.pdf

6.2.6 EPA Method 907.0: Actinide Elements in Drinking Water - Thorium, Uranium, Neptunium, Plutonium, Americium and Curium

Analyte(s)	CAS RN
Neptunium-237	13994-20-2
Thorium-227	15623-47-9
Thorium-228	14274-82-9
Thorium-230	14269-63-7
Thorium-232	7440-29-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Alpha emitting actinide elements in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water samples.

Description of Method: Actinide elements are concentrated by coprecipitation using ferric hydroxide. The ferric hydroxide is dissolved and thorium, neptunium, plutonium, americium and curium are coprecipitated with bismuth phosphate. The bismuth phosphate is dissolved in 8M hydrochloric acid and plutonium and neptunium are extracted in tri-isooctylamine (TIOA). The thorium is separated from americium and curium by extraction with trioctylphosphine oxide (TOPO). All separated and purified elements are coprecipitated on lanthanum fluoride and alpha counted.

Special Considerations: The technical contacts in Section 4.0 should be consulted regarding use of alpha spectrometry for analysis of samples prepared using this method to detect and measure specific isotopes. Ammonium ions interfere in the precipitation of neptunium with ferric hydroxide. If ammonium ions are present, adding sodium hydroxide to raise the pH should result in complete recovery of neptunium. Chelating agents, which are present in some decontamination agents, will interfere to varying extents by totally or partially comp1exing actinide elements. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well. When chelating agents are present, alternate methods, such as coprecipitation from acid solutions (Section 6.2.26), should be considered. Clays that are present in some decontamination agents can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interferences.

Source: U.S. EPA, EMSL. 1980. "Method 907.0: Actinide Elements in Drinking Water - Thorium, Uranium, Neptunium, Plutonium, Americium and Curium." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032.
https://nepis.epa.gov/Exe/ZyPDF.cgi/30000QHM.PDF?Dockey=30000QHM.PDF

6.2.7 EPA Method EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Isotopic plutonium, uranium and thorium, together or individually, in soil, water, air filters, urine or ashed residues of vegetation, animal tissues and bone
Method Selected for: This method has been selected for confirmatory analysis of plutonium-238 and -239 in drinking water samples.

Description of Method: This method is appropriate for the analysis of isotopic plutonium, uranium and thorium, together or individually, by alpha spectrometry. Plutonium-236, uranium-232 and thorium-234 tracer standards are added for the determination of chemical yields. Samples are decomposed by nitric-hydrofluoric acid digestion or ignition to assure that all of the plutonium is dissolved and chemically separated from the sample by coprecipitation with sodium and ammonium hydroxide, anion exchange and electrodeposition. The residues are dissolved in dilute nitric acid and successive sodium and ammonium hydroxide precipitations are performed in the presence of boric acid to remove fluoride and soluble salts. The hydroxide precipitate is dissolved, the solution is pH-adjusted with hydrochloric acid, and plutonium and uranium are adsorbed on an anion exchange column, separating them from thorium. Plutonium is

eluted with hydrobromic acid. The actinides are electrodeposited on stainless steel discs from an ammonium sulfate solution and subsequently counted by alpha spectrometry. This method is designed to detect environmental levels of activity as low as 0.02 pCi per sample. To avoid possible cross-contamination, sample aliquot activities should be limited to 25 pCi or less.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Some chelators also can tightly complex barium that may be present in the sample causing interferences when analyzing for plutonium-238. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate radionuclides out of solution prior to analysis.
- The presence of higher valence anions can lead to lower yields when using the evaporation option, due to competition with active sites on the resin used to collect the radionuclides.

Source: U.S. EPA, EMSL. 1979. "EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue." *Radiochemical Analytical Procedures for Analysis of Environmental Samples*. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-emsl-33.pdf

6.2.8 EPA Method Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative analysis
Technique: Liquid scintillation

Method Developed for: Phosphorus-32 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: A 100-mL water sample is filtered and phosphate carrier is added to the filtered sample. The solution is then passed through a cation exchange resin, followed by a Diphonix resin, to remove interferences from cation radionuclides. The eluent is treated with a mixture of 10 mL of 30% hydrogen peroxide and 10 mL of concentrated nitric acid, reduced to approximately 2–5 mL by heating, and quantitatively transferred to a liquid scintillation vial for counting. The Čerenkov photons from the P-32 beta (1710 keV, E_{max}) decay are detected using a calibrated liquid scintillation counter (LSC). Following counting of the sample, an aliquot of the final solution is used for yield determination by the inductively coupled plasma-atomic emission spectrometry (ICP-AES) method.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water. Organic compounds containing oxygen, reductants, halogenated compounds or elevated levels of nitrates or nitromethane, such as those contained in some decontamination agents, can cause chemical quenching.

Chemical and color quenching compounds, such as dyes and pigments, also contained in some decontamination agents, can have a significant impact when using liquid scintillation methods. This method also can be impacted by high levels of phosphates or phosphorus compounds.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA/600/R-11/181. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.9 EPA Method R4-73-014: Radioactive Phosphorus

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Phosphorus-32 in nuclear reactor solutions
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid-phase samples, and for confirmatory analysis of drinking water samples.

Description of Method: 200 mL or less of a water sample is acidified with nitric acid and carriers of phosphorus (standardized), cobalt, zirconium, silver and manganese are added. Hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide, and the hot solution is filtered through filter paper. Carriers of cobalt and zirconium are added to the filtrate, and the hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide. The solution is filtered and the hydroxides are discarded. The filtrate is acidified with hydrochloric acid, and phosphorous is precipitated as magnesium ammonium phosphate by the addition of a magnesium mixture and ammonium hydroxide. The magnesium ammonium phosphate is collected on a tared filter, dried, and weighed to determine the chemical yield. The precipitate is mounted and beta counted with a gas-flow proportional counter.

Special Considerations: Chelating compounds, such as those present in some decontamination agents, can compromise the collection of scavenging carriers that are added to the sample solution prior to analysis by preventing them from being precipitated out of solution, affecting the chemical yield of phosphorus-32. This method also can be impacted by the presence of high levels of phosphates or phosphorus compounds.

Source: U.S. EPA. EMSL. 1980. "Method R4-73-14: Radioactive Phosphorus." *Prescribed Procedures for Radiochemical Analysis of Nuclear Reactor Solutions*. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-r4-73-014.pdf

6.2.10 EPA Method: Determination of Radiostrontium in Food and Bioenvironmental Samples

Analyte(s)	CAS RN
Strontium-89	14158-27-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Strontium-89 and strontium-90 in food, vegetation and tissue samples
Method Selected for: This method has been selected for qualitative analysis of strontium-89 in wipes and air filters and confirmatory analysis of strontium-89 in wipes, air filters, soil and sediment, and vegetation.

Description of Method: This method is used for the determination of strontium-89 and strontium-90 in various bio-environmental samples. A sample of 10 g or less is placed in a nickel crucible. Barium and strontium (standardized) carriers are added to the sample. Sodium hydroxide pellets and anhydrous sodium carbonate are added and mixed, and the sample is fused as a carbonate. The strontium-calcium carbonates are dissolved in hydrochloric acid, complexed with di-sodium EDTA, and passed through a cation column where the strontium is absorbed and the complexed calcium passes through. The strontium is eluted from the column and precipitated as a carbonate. The strontium carbonate is weighed and mounted on a planchet for beta counting with a low background gas-flow alpha beta counter. The chemical yield is determined gravimetrically, using calculations provided in the method.

Special Considerations: This method was developed for analysis of food, vegetation and tissue. Additional laboratory development and testing is necessary for application to soil, sediment, air filters and wipes. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: U.S. EPA, National Environmental Research Center. 1975. "Determination of Radiostrontium in Food and Bioenvironmental Samples." *Handbook of Radiochemical Methods*. Washington, DC: U.S. EPA. EPA-680/4-75-001. http://www.epa.gov/sites/production/files/2015-06/documents/radiostrontium_in_food.pdf

6.2.11 EPA Method: Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: The method is based on a sequence of two chromatographic extraction resins. Americium is concentrated, isolated and purified by removing interfering radionuclides as well as other components of the sample in order to prepare the americium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to use of the extraction resins, the water sample is filtered as necessary to remove any insoluble fractions, equilibrated with americium-243 tracer, and concentrated by evaporation or calcium phosphate precipitation. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. The presence of higher valence anions such as phosphates can lead to lower yields when using the evaporation option in this method, due to competition with active sites on the resin. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison the extraction resin used in this method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Reducing or oxidizing compounds can result in lower measured concentrations when using this method, which requires specific valence states for radionuclides.
- Compounds, such as clays that are also present in some decontamination agents, contain iron, magnesium and calcium, which can be released as ions via ion exchange in the presence of radionuclides, and cause interference when analyzing water samples.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate radionuclides out of solution prior to analysis.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-10-001a.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.12 EPA Method: Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential use of two chromatographic extraction resins to isolate and purify plutonium by removing interfering radionuclides as well as other components of the matrix in order to prepare the plutonium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to using the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with plutonium-242 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds such as clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange, and cause interference when analyzing water samples.

- High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison the extraction resins used in this method.
- Higher valence anions may lead to lower yields when using the evaporation option due to competition with active sites on the resin used to collect the radionuclides.
- The presence of fluoride can precipitate out plutonium prior to measurement.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-10-001b. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.13 EPA Method: Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Radium-223	15623-45-7
Radium-226	13982-63-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in water
Method Selected for: This method has been selected for qualitative analysis of radium-226 in drinking water samples and for the qualitative and confirmatory analysis of radium-223 in drinking water, aqueous/liquid-phase, soil and sediment, surface wipes, air filters and vegetation samples.

Description of Method: A known quantity of radium-225 is used as the yield determinant in this analysis. The sample is initially digested using concentrated nitric acid, followed by volume reduction and conversion to the chloride salt using concentrated hydrochloric acid. The solution is adjusted to a neutral pH and batch equilibrated with manganese resin to separate radium from any radioactive and/or non-radioactive matrix constituents. Further selectivity is achieved using a column containing Diphonix resin. The radium (including radium-226 and -223) eluted from the column is prepared for counting by microprecipitation with barium sulfate. Low-level measurements are performed by alpha spectrometry. The activity measured in the radium-226 and -223 region of interest is corrected for chemical yield based on the observed activity of the alpha peak at 7.07 mega-electron volts [MeV].

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. Although the method has not been validated in sample types other than water, it is likely to be applicable to the additional sample types for qualitative and confirmatory analyses of radium-223. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Permanganate and permanganic acid can be reduced to insoluble manganese (IV) oxide, which could remove radium.
- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resin used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-10-001c.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.14 EPA Method: Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: Strontium is isolated from the sample matrix and purified from potentially interfering radionuclides and matrix constituents using a strontium-specific, rapid chemical separation procedure. The sample is equilibrated with strontium carrier and concentrated by coprecipitation with strontium/barium carbonate. If insoluble residues are noted during acid dissolution steps, the residue and precipitate mixture is digested in 8M nitric acid to solubilize strontium. The solution is passed through a chromatography column that selectively retains strontium while allowing most interfering radionuclides and matrix constituents to pass through to waste. If present in the sample, residual plutonium and several interfering tetravalent radionuclides are stripped from the column using an oxalic acid/ nitric acid rinse. Strontium is eluted from the column with 0.05M nitric acid and taken to dryness in a tared, stainless steel planchet. The planchet containing the strontium nitrate precipitate is weighed to determine the strontium yield. The sample test source is promptly counted on a gas flow proportional counter to determine the beta emission rate, which is used to calculate the total radiostrontium activity.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. High levels of radioactive cesium or cobalt (>1,000 times the activity of strontium being measured) may not be completely removed during ion exchange and can cause interferences. Chelating compounds, such as those present in some decontamination agents, can compromise the collection of strontium prior to analysis, by preventing it from being trapped on an ion exchange column or from being precipitated out of solution. Compounds containing carbonate, fluoride, phosphate or sulfate, also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of strontium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-10-001d.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.15 EPA Method: Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Uranium-234, -235 and -238 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential elution of interfering radionuclides as well as other components of the sample matrix by extraction chromatography to isolate and purify uranium for counting by alpha spectrometry. The method utilizes vacuum assisted flow to improve the speed of the separations. Prior to the use of the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with uranium-232 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.
Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water. Higher valence anions may lead to lower yields when using the evaporation option due to competition with active sites on the resin. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interference in the analysis of water. High levels of iron, manganese, calcium or magnesium can also impact exchange site availability and/or poison the extraction resins used in this method.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate uranium out of solution.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-10-001e.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.16 EPA Method: Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: This method has been selected for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter material and deposited particulates. Glass-fiber filters (the siliceous filter as well as deposited silicates) are dissolved with direct application of hydrofluoric acid. The addition of nitric and hydrochloric acids facilitates dissolution of remaining solids. The sample digestate is taken to dryness and re-dissolved in nitric acid. Filters composed of organic materials, such as cellulose or polypropylene, are dry ashed in a 450°C muffle furnace to destroy the organic filter material, then processed through the acid dissolution steps referenced above for non-organic filter material. Once sample dissolution is complete, it is re-dissolved in nitric acid solution. The sample is then processed for specific analyte determination using one of the following rapid methods contained in *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events.*
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.11)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.12)
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.13)
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.14)
- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.15)

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited above. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may require repeated application of the digestion procedure to cause a complete dissolution of the particulates. If refractory constituents are suspected in the sampled particulates or the acidic digestion procedure is otherwise deemed to be ineffective because of refractory residuals or constituents, the alternate *Rapid*

Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events (Section 6.2.17) should be considered for sample preparation.

Some concrete or brick materials can have native concentrations of uranium, radium, thorium, strontium or barium, all of which can have an effect on the chemical separations used following sample fusion. In some cases (e.g., radium or strontium analysis), elemental analysis of the digest prior to chemical separation may be necessary to determine native concentrations of carrier elements. Lanthanum is used in this method to pre-concentrate actinides, along with lanthanum (III) fluoride, to eliminate matrix interferences including silica, which can cause column flow problems. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-12-009. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.17 EPA Method: Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: This method has been selected for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter or swipe material and the deposited particulates. Glass-fiber media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. The resulting fusion cake is dissolved in hydrochloric acid. Filters composed of organic materials, such as cellulose or polypropylene, are charred in a crucible to destroy the organic filter material. The resulting charred media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. This resulting fusion cake is also dissolved in hydrochloric acid. Once sample fusion is complete and the fusion cake is dissolved in hydrochloric acid, the sample is processed for specific analyte determination

using one of the following rapid methods:

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.11)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.12)
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.13)
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.14)
- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.15)

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may require repeated application of the digestion procedure to cause a complete dissolution of the particulates.

Some surface materials (e.g., concrete or brick) can contain native concentrations of uranium, radium, thorium, strontium or barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In some cases (e.g., radium or strontium analysis), elemental analysis of the digest prior to chemical separation may be necessary to determine concentrations of carrier elements. Trace levels of radium-226 may be present in sodium carbonate used in the pre-concentration step used in this method. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R-12-008. https://www.epa.gov/sites/default/files/2015-06/documents/air_filter_dissolution_by_na_carbonate_fusion_402-r-12-008_10-22-12.pdf

6.2.18 Rapid Method for Radium in Soil Incorporating the Fusion of Soil and Soil-Related Matrices with the Radioanalytical Counting Method for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil and sediment samples.

Description of Method: This method is based on the complete fusion of a representative, finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C in an appropriate vessel prior to fusion, then dissolved

in a crucible with hydrofluoric acid and evaporated to dryness. Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is warmed under a flame until a reaction initiates. The crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in hydrochloric acid, and transferred to a digestion container while rinsing the crucible with 6M hydrochloric acid. The barium content is determined for a small aliquot of the dissolved flux by adding sufficient amount of barium so that the final mass (native plus added) is not more than 90 µg and then analyzing by ICP-AES. A manganese (IV) solution and phenolphthalein indicator are added to this mixture, and the pH is adjusted with sodium hydroxide until the solution turns pink. Hydrogen peroxide is slowly added forming insoluble manganese (II) oxide. The manganese (IV) oxide precipitate is centrifuged, rinsed with water and dissolved in a manganese (IV) oxide stripping agent. Ascorbic acid is added and the solution is passed through a resin column, rinsing with hydrochloric acid. The rinse solution is collected, ammonium sulfate and isopropanol are added, and the solution is placed in a cold water ultrasonic bath for 20 minutes, after which it is filtered, rinsing with a solution of ammonium sulfate in isopropanol. The filter is then placed in a Petri dish, dried, and stored for at least 24 hours. The sample is then counted by alpha spectrometry.

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation, as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used when digesting samples with hydrofluoric acid. If platinum crucibles are not available, effective alternate methods are available that use zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

In some cases, elemental analysis of the digest prior to chemical separations may be necessary to determine native concentrations of carrier elements present in the sample. Trace levels of radium-226 might be present in the sodium carbonate used in the pre-concentration step.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Radium in Soil Incorporating the Fusion of Soil and Soil-Related Matrices with the Radioanalytical Counting Method for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-635.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.19 Rapid Method for Fusion of Soil and Soil-Related Matrices Prior to Americium, Plutonium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238, plutonium-239, uranium-234, uranium-235 and uranium-238 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil and sediment samples.

Description of Method: The method is based on the complete fusion of a representative finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C in an appropriate vessel prior to fusion. The sample is dissolved in a crucible with hydrofluoric acid, and evaporated to dryness on a hotplate at medium to high heat (~300°C). Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is warmed under a flame until a reaction initiates. The crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in nitric acid. The dissolved sample is transferred to an appropriately sized beaker, and the crucible is rinsed with nitric acid to ensure a quantitative transfer of material. The sample is then processed using one of the following methods:

- Rapid Radiochemical Method for Americium-241 in Water (Section 6.2.11)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water (Section 6.2.12)
- Rapid Radiochemical Method for Isotopic Uranium in Water (Section 6.2.15)

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used when digesting samples with hydrofluoric acid. If platinum crucibles are not available, effective alternate methods are available that use zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Fusion of Soil and Soil-Related Matrices Prior to Americium, Plutonium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-636, EPA-600-R-12-637 and EPA-600-R-12-638.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.20 Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative analysis

Technique: Beta counting

Method Developed for: Strontium-90 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil and sediment samples.

Description of Method: The method is based on the complete fusion of a representative, finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C prior to fusion. The sample is dissolved in a crucible with hydrofluoric acid and evaporated to dryness on a hotplate at medium to high heat (~300°C). Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is heated under a low flame; initial heating may produce a vigorous reaction. After the initial reaction subsides, the crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in nitric acid. Calcium solution and phenolphthalein indicator are added to this mixture, and the pH is adjusted to 8.3 with sodium hydroxide. The sample will become pinkish-orange due to the indicator color change and the formation of hydroxide precipitate. Sodium carbonate and heat are added to complete precipitation. After cooling and allowing the precipitate to settle, the supernatant is decanted and the precipitate is transferred to a centrifuge tube and dissolved in nitric acid. The sample is then processed for strontium-90 determination using *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Restoration Following Homeland Security Events* (Section 6.2.14).

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used in this method when digesting samples with hydrofluoric acid. If platinum crucibles are not available, an effective, alternate method is available that uses zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-640.
https://www.epa.gov/sites/default/files/2015-06/documents/soil_dissolution_by_fusion_for_sr-90_09-17-12_epa-600-r-12-640.pdf

6.2.21 Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices Prior to Plutonium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in water and air filters
Method Selected for: This method has been selected as a pre-treatment technique supporting analysis of refractory radioisotopic forms of plutonium in drinking water and air filters using the following qualitative techniques:

- Rapid methods for acid or fusion digestion (Sections 6.2.16 and 6.2.17)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium 239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)

Description of Method: This method is a pre-treatment technique for qualitative analysis of water and air filters, and has been validated together with the chemical separation and analysis process described in EPA's *Rapid Radiochemical Method for Plutonium-238 and Plutonium 239/240 in Building Materials for Environmental Remediation Following Radiological Incidents* (Section 6.3.5). The method is based on total dissolution of radioisotope thermoelectric generator (RTG). Air filters are fused using rapid sodium hydroxide/sodium peroxide at 700°C. For water samples, refractory RTG particles are collected on a 0.45µm filter using a vacuum, and RTG activity in the filtrate is preconcentrated using calcium phosphate precipitation. Solid fractions and filtrate fractions are processed separately by fusing with sodium hydroxide/sodium peroxide prior to subsequent chemical separation and alpha spectrometric analysis. Pre-concentration steps are needed to eliminate the alkaline fusion matrix and collect the radionuclides. Plutonium is separated from the fusion matrix using a lanthanum/calcium fluoride matrix removal step in preparation for separation and analysis using the rapid separation method cited above. Assuming a 68 m^3 air volume, the method is capable of meeting a required minimum detectable concentration (MDC) of 0.003 pCi/m^3 or below for air filters (240-minute count time) and an uncertainty of 1.9 pCi/filter at and below the analytical action level of 15.0 pCi/filter (360-minute count time). Assuming a 1-L volume and 360-minute count time, the method is capable of satisfying an uncertainty of 2.1 pCi/L at and below an analytical action level of 16.3 pCi/L, and meeting a required MDC of 0.23 pCi/L for water samples (filtered solids, filtrate, or combined result) with a 240-minute count time.

Special Considerations: Organic-based materials, such as cellulose nitrate or cellulose acetate filters, may react vigorously upon addition of peroxide or during charring steps. Wet ashing with nitric acid and hydrogen peroxide is needed to destroy organic constituents prior to fusion. Samples with elevated activity or samples that require multiple analyses may need to be split after dissolution. Reducing or oxidizing compounds, such as those present in some decontamination agents, can impact this method, which requires specific valence states for radionuclides. All plutonium must be reduced to plutonium (+3 or +4) before isotopic exchange with the tracer can be achieved with reasonable certainty. Additionally, only plutonium (+3 or +4) will precipitate in the lanthanum fluoride/calcium fluoride pre-concentration step. Although peroxide may reduce plutonium +6 to +4, the valence must be controlled with certainty. Valence controls also ensure that plutonium will be present in the plutonium +4 form prior to separation. Although this method was validated using plutonium-242 tracer, plutonium-236 tracer can be used assuming it can be obtained with sufficient purity.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices Prior to Plutonium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.22 EPA Method: Rapid Radiochemical Method for Californium-252 in Water, Air Particulate Filters, Swipes and Soil for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Californium-252	13981-17-4

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Californium-252 in water, air particulate filters, swipes and soil samples

Method Selected for: This method has been selected for confirmatory analysis of californium-252 in drinking water, aqueous/liquid-phase, air filter, surface wipes and soil matrices.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify californium by removing interfering radionuclides as well as other matrix components. The method utilizes vacuum-assisted flow to improve the speed of the separations. Americium-243 tracer equilibrated with the sample is used as a yield monitor.

- Water samples are concentrated using a calcium phosphate coprecipitation. The calcium phosphate precipitate is dissolved in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Glass-fiber or cellulose-based air particulate filter samples are wet ashed with repeated additions of nitric and hydrofluoric acids and hydrogen peroxide. The residues are treated with nitric-boric acid, and dissolved in a load solution containing 3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Cotton-twill swipe and organic-polymer-based air particulate filter samples are dry ashed in a beaker for 30−60 minutes using a ramped program to minimize the risk of flash-ignition. The residue is transferred to a polytetrafluoroethylene (PTFE) beaker with nitric acid and hydrogen peroxide, digested with hydrofluoric acid, and taken to dryness. The residues are wet ashed with nitric acid and hydrogen peroxide and taken to dryness before being treated with nitric-boric acid and dissolved in a load solution containing 3M nitric acid/1M aluminum nitrate for chemical separations.
- Soils are finely ground before being fused with sodium hydroxide in zirconium crucibles. The fusion cake is dissolved in water and californium preconcentrated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. The fluoride precipitate is dissolved with nitric-boric acid and diluted in nitric acid and aluminum nitrate to yield a load solution containing ~3M nitric acid/1M aluminum nitrate.

Extraction chromatography resins (TEVA + DGA resins [Eichrom Technologies, Lisle, IL, or equivalent]) are then used to isolate and purify californium and americium by removing interfering radionuclides and other matrix components. Following chemical separation of curium and americium, the sample test source is prepared by microprecipitation with cerium (III) fluoride.

Water*:* This method is capable of achieving a required method uncertainty for californium-252 of 2.0 pCi/L at an analytical action level of 15.3 pCi/L and a required MDC of 1.5 pCi/L, using a sample volume of 0.2 L and count time of at least 4 hours.

Air Particulate Filter: This method is capable of achieving a required method uncertainty for californium-252 of 0.57 pCi/filter at an analytical action level of 4.37 pCi/filter and a required MDC of 0.44 pCi/filter, using a sample aliquant of one filter and count time of at least 4 hours.

Swipe or Organic-Polymer-Based Air Particulate Filter: This method is capable of achieving a required method uncertainty for californium-252 of 0.12 pCi/swipe or filter at an analytical action level of 0.12 pCi/ swipe or filter and a required MDC of 0.15 pCi/ swipe or filter, using a sample aliquant of one swipe or filter and count time of at least 4 hours.

Soil: This method is capable of achieving a required method uncertainty for californium-252 of 0.18 pCi/g at an analytical action level of 1.38 pCi/g and a required MDC of 0.14 pCi/g, using a sample weight of 1 g and count time of at least 4 hours.

Special Considerations: Alpha emissions from californium-250 fall in the same region as californium-252 and cannot be differentiated from those of californium-252 using alpha spectrometry. Measurements should be reported in terms of the activity of californium-250/252. Since alpha spectrometry does not differentiate between californium-250 and californium-252, decay corrections based on the half-life of californium-252 will impart a positive bias to results as mixtures age. The effect can be minimized by keeping the time between the activity reference date (i.e., collection or standard reference date) short, or by reporting the activity at the time of the measurement.

Other radionuclides (or their short-lived progeny) that emit alpha particles that are isoenergetic with californium-252 (e.g., bismuth-212 at 6.1 MeV supported by thorium-228 and/or radium-224) must be chemically separated to prevent positive interference with the measurement. This method effectively separates these radionuclides.

The use of the americium-243 tracer assumes that both californium and americium are removed from the column at the time of elution. The separation scheme is designed to ensure that nitrates and lanthanum will not interfere with this elution. High levels of calcium in soil samples can have an adverse impact on the retention of californium and americium on the DGA resin. The method is designed to minimize calcium interference and enhance californium and americium affinity by increasing the nitrate concentration in the load and initial rinse solutions. Non-radiological anions, including fluoride and phosphate, can complex californium and americium and lead to depressed yields. Boric acid added to the load solution will complex residual fluoride ions, while aluminum in the load solution will complex phosphate ions.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2017. "[Validation of] Rapid Radiochemical Method for Cf-252 in Water, Air Particulate Filters, Swipes and Soils for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.23 EPA Method: Rapid Radiochemical Method for Curium-244 in Water Samples for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Curium-244 in water samples
Method Selected for: This method has been selected for confirmatory analysis of curium-244 in water samples.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify curium by removing interfering radionuclides and other matrix components and preparing the curium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. An americium-243 tracer is equilibrated with the water sample and used as a yield monitor. Following chemical separation of curium and americium, the sample test source is prepared by microprecipitation with cerium (III) fluoride. Alpha emissions from the source are measured using an alpha spectrometer and used to calculate the activity of curium-244 in the sample.

Using a 0.2 L sample and a count time of four hours, this method is capable of achieving an uncertainty of 2.0 pCi/L californium-252 at an analytical action level of 15 pCi/L and a required MDC of 1.515 pCi/L.

Special Considerations: The alpha emissions from curium-243 fall in the same region as curium-244 and cannot be differentiated using alpha spectrometry. Although curium-243 and curium-244 alpha emissions overlap, monitoring the region of the spectrum between 5.8 and 6.0 MeV for less intense emissions of curium-243 can qualitatively indicate the presence of curium-243 in a sample. Alpha spectrometry measurements that show activity in the region of interest for curium-244 should be reported as curium-243/244. Americium and californium are chemical analogs of curium in the separations scheme used for this analysis. Several isotopes of californium or americium emit alpha particles within the region of interest for curium-244. These include californium-249 and californium-251. If high levels of californium could be present in samples, alpha spectrometry results should be monitored for other isotopes of californium. Radionuclides of other elements (or their short-lived progeny) that emit alpha particles which are isoenergetic with curium-244 (e.g., thorium-227 or actinium-225 5.8 MeV) must be chemically separated using the method procedures to prevent positive interference.

Non-radiological anions that can complex curium, including fluoride and phosphate, can lead to depressed yields. Aluminum in the load solution will complex both fluoride and residual phosphate. High levels of calcium can have an adverse impact on curium and americium retention on DGA resin. Calcium retention is minimized, and curium and americium affinity is enhanced, by increasing nitrate concentrations in the load and initial rinse solutions. A dilute nitric acid rinse is performed on DGA resin to remove calcium that could otherwise end up in the sample test source as the fluoride. For samples containing elevated concentrations of calcium, it may be advisable to increase the volume of this rinse step slightly to better remove calcium ions and possibly improve alpha peak resolution.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Radiochemical Method for Curium-244 in Water Samples for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-001.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.24 EPA Method: Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil

Analyte(s)	CAS RN
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Curium-244 in air particulate filters, swipes and soil samples
Method Selected for: This method has been selected for confirmatory analysis of curium-244 in air particulate filters, swipes and soil matrices.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify curium by removing interfering radionuclides and matrix components in order to prepare the curium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. An americium-243 tracer is equilibrated with the sample as a yield monitor.

- Glass-fiber or cellulose-based air particulate filter samples are wet ashed with repeated additions of nitric acid and hydrofluoric acid, and hydrogen peroxide. The residues are treated with nitric-boric acid and dissolved in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Cotton-twill swipe and organic-polymer-based air particulate filter samples are dry ashed in a beaker for 30-60 minutes using a ramped program to minimize the risk of flash-ignition. The residue is transferred to a PTFE beaker with nitric acid and hydrogen peroxide, digested with hydrofluoric acid, and taken to dryness. The residues are then wet ashed with nitric acid and hydrogen peroxide and taken to dryness before being treated with nitric-boric acid and dissolved in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Soils are finely ground before being fused with sodium hydroxide in zirconium crucibles. The fusion cake is dissolved in water and curium preconcentrated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. The fluoride precipitate is dissolved with nitric-boric acid and diluted in nitric acid and aluminum nitrate to yield a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.

Extraction chromatography resins are then used to isolate and purify curium by removing interfering radionuclides and other matrix components. The method utilizes vacuum-assisted flow to improve the speed of the separations. Following chemical separation of curium and americium, the sample test source (STS) is prepared by microprecipitation with cerium (III) fluoride.

Air Particulate Filters: This method is capable of achieving a required method uncertainty for curium-244 of 1.4 pCi/filter at an analytical action level of 10.5 pCi/filter and a required MDC of 0.25 pCi/filter, using a sample aliquant of one filter and a count time of at least four hours.

Swipes (or Organic-Polymer-Based Air Particulate Filters): This method is capable of achieving a required method uncertainty for curium-244 of 0.051 pCi/swipe at an analytical action level of 0.39 pCi/swipe and a required MDC for of 0.065 pCi/swipe, using one swipe and a count time of at least four hours.

Soil: This method is capable of achieving a required method uncertainty for curium-244 of 0.66 pCi/g at an analytical action level of 5.09 pCi/g and a required MDC of 0.66 pCi/g, using a sample weight of 1 gram and a count time of at least four hours.

Special Considerations: The alpha emissions from curium-243 fall in the same region as curium-244 and cannot be differentiated using alpha spectrometry. Although curium-243 and curium-244 alpha emissions overlap, monitoring the region of the spectrum between 5.8 and 6.0 MeV for less intense emissions of curium-243 can qualitatively indicate the presence of curium-243 in a sample. Alpha spectrometry measurements that show activity in the region of interest for curium-244 should be reported as curium-243/244. Americium and californium are chemical analogs of curium in the separations scheme used for this analysis. Several isotopes of californium or americium emit alpha particles within the region of interest for curium-244. These include californium-249 and californium-251. If high levels of californium could be present in samples, alpha spectrometry results should be monitored for other isotopes of californium. Radionuclides of other elements (or their short-lived progeny) that emit alpha particles which are isoenergetic with curium-244 (e.g., thorium-227 or actinium-225 5.8 MeV) must be chemically separated using the method procedures to prevent positive interference.

Non-radiological anions that can complex curium, including fluoride and phosphate, can lead to depressed yields. Aluminum in the load solution will complex both fluoride and residual phosphate. High levels of calcium can have an adverse impact on curium and americium retention on DGA resin. Calcium retention is minimized, and curium and americium affinity is enhanced, by increasing nitrate concentrations in the load and initial rinse solutions. A dilute nitric acid rinse is performed on DGA resin to remove calcium that could otherwise end up in the sample test source as the fluoride. For samples containing elevated concentrations of calcium, it may be advisable to increase the volume of this rinse step slightly to better remove calcium ions and possibly improve alpha peak resolution.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-004. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.25 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in building materials
Method Selected for: This method is selected for confirmatory analysis of radium-226 in surface wipes and air filters (also see Section 6.3.2 for application of this method to building materials).

Description of Method: A known quantity of radium-225 is used as the yield tracer in this analysis. Samples are fused using procedures in *Rapid methods for acid or fusion digestion* (Sections 6.2.16 and 6.2.17), and the radium isotopes are removed from the fusion matrix using a carbonate precipitation step.

The sample is acidified and loaded onto a cation exchange resin to remove interferences such as calcium, and radium is eluted from the cation resin with 8M nitric acid. After evaporation of the eluate, the sample is dissolved in a minimal amount of 3M nitric acid and passed through Sr Resin to remove any barium. This solution is then evaporated to dryness, redissolved in 0.02M hydrochloric acid, and passed through Ln Resin to remove interferences such as calcium, and to remove the initial actinium-225. The radium (including radium-226) is prepared for counting by micro-precipitation with barium sulfate. Activity measured in the radium-226 region of interest is corrected for chemical yield based on observed activity of the alpha peak at 7.07 MeV (astatine-217, the third progeny of radium-225).

This method is suited for low-level measurements for radium-226 using alpha spectrometry and is capable of satisfying a method uncertainty of 0.83 pCi/g at an analytical action level of 6.41 pCi/g, using a sample aliquant of ~1g and count time of at least eight hours.

Special Considerations: Depending on actual spectral resolution, method performance may be compromised if samples contain high levels of other radium isotopes (e.g., ~3 times the radium-226 activity concentration) due to ingrowth of interfering decay progeny. Calcium, iron (+3 oxidation state), and radionuclides with overlapping alpha energies, such as thorium-229, uranium-234, and neptunium-237, will interfere if they are not removed effectively. Delaying the count significantly longer than one day may introduce positive bias in results near the detection threshold due to the decay progeny from the radium 225 tracer. If radium-226 measurements close to detection levels are required and sample counting cannot be performed within ~36 hours of tracer addition, the impact of tracer progeny tailing into the radium-226 may be minimized by reducing the amount of the tracer that is added to the sample. This will aid in improving the signal-to-noise ratio for the radium-226 peak by minimizing the amount of tailing from higher energy alphas of the radium-225 progeny. If actinium-225 is present prior to the final separation time and the flow rate through the column is too fast (>1.5 drops/second), then actinium-225 will break through the resin, resulting in a high bias in the tracer yield. Additional information regarding procedures to remove or minimize interferences is provided in Section 4.0 of the method. Clays that are present in some decontamination agents can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interferences. High levels of iron, manganese, calcium or magnesium might have an impact on exchange site availability and or poisoning of the extraction resins used. Chelators, also present in some decontamination agents, can tightly complex barium, calcium, iron and magnesium that may be present in the sample, causing interferences when analyzing for radium-226.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-002. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.26 EPA Method: NAREL Standard Operating Procedure for Actinides in Environmental Matrices by Extraction Chromatography

Analyte(s)	CAS RN
Neptunium-237	13994-20-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Thorium-227	15623-47-9
Thorium-228	14274-82-9
Thorium-230	14269-63-7
Thorium-232	7440-29-1
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry / Beta counting (analysis of tracer)
Method Developed for: Americium-241; neptunium-237; plutonium-238 and -239; thorium-227, -228, -230 and -232; and uranium-234, -235 and -238 in water, soil, vegetation, air filters, swipes and tissue
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid-phase, soil and sediment, surface wipes, air filters and vegetation samples.

- Standard Methods 7500-U B (Section 6.2.56) and 7500-U C (Section 6.2.57) should be used for qualitative (7500-U B) and confirmatory (7500-U C) analysis of uranium-234, -235 and -238 in aqueous/liquid-phase samples.
- ASTM Method D3084-20 (Section 6.2.48) should be used for qualitative analysis of plutonium-238 and -239 in aqueous/liquid-phase samples.
- EPA NAREL Rapid Method for Fusion of Soil and Soil-Related Matrices (Section 6.2.19) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238 in soil and sediment samples.
- EPA NAREL Rapid Methods for Acid or Fusion Digestion (Sections 6.2.16 and 6.2.17) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238 in surface wipes and air filters.
- DOE SRS Actinides and Sr-89/90 in Vegetation (Section 6.2.41) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238 in vegetation.
- EML HASL-300 Methods Am-06-RC (Section 6.2.29) and U-02-RC (Section 6.2.37) should be used for confirmatory analysis of plutonium -238 and -239 and uranium-234, -235 and -238 in vegetation, respectively.

Description of Method: This method involves the use a tandem arrangement of cartridges containing extraction chromatographic resins connected in series, which effectively separate and isolate americium, plutonium, thorium, uranium and neptunium from a variety of environmental matrices. The oxidation states of the elements of interest in the load solution are as follows: americium (III), neptunium (IV), plutonium (III), thorium (IV) and uranium (VI). The sample is first loaded onto a TEVA-resin (Eichrom Technologies, Lisle, IL, or equivalent) cartridge. Any thorium or neptunium present in the sample will be retained on the cartridge. The effluent passes through this cartridge and onto a transuranic (TRU)-resin cartridge, which will retain any americium, plutonium or uranium. The tandem cartridge arrangement is then separated, and the elements of interest are selectively eluted, then co-precipitated as a fluoride complex and radio-assayed by alpha-particle spectrometry.

Special Considerations: Prior to adding americium-243 or neptunium-239 tracers, the sample should be analyzed for native neptunium-239 by the appropriate method (EPA Method 901.1 for drinking water;

Standard Method 7120 for aqueous/liquid-phase; and HASL-300 Method Ga-01-R for surface wipes, air filters and vegetation).

If present, iron (III) can interfere with retention of the actinides on the TRU resin and must be reduced with ascorbic acid to iron (II) so that it will not interfere with desired chemical reactions. Phosphates, sulfates, and oxalates can cause interferences by forming insoluble complexes with actinides. These anions can be complexed to aluminum (III) so that they do not interfere with the analysis. In fact, the presence of aluminum (III) actually increases the retention factor of americium to the TRU resin. There may be instances when increasing the aluminum (III) concentration in the nitric acid/aluminum nitrate load solution can improve radiochemical separation and recovery.

Neptunium and americium cannot be determined sequentially, and each must be analyzed using a separate sample aliquot. The tracer used for americium analysis is americium-243, while the one used for neptunium analysis is neptunium-239, which is in equilibrium with americium-243. The neptunium-239 yield is determined by beta counting using approximately the amount of neptunium-239 equivalent to ~50 dpm of americium-243. The calcium phosphate preparation option described may give low plutonium recoveries in unpreserved or weakly preserved samples. Until the calcium phosphate procedure is revised, analysts must use the evaporation/digestion option when preparing water samples for plutonium analysis.

High levels of iron, manganese, calcium or magnesium, which are present in some decontamination agents, can impact exchange site availability and/or poison the extraction resins. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences.
- Compounds containing carbonate, fluoride, hydroxide, or phosphate, present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis of water samples, resulting in a lesser amount of polonium in cases where an aliquot of water sample is transferred prior to the precipitation step.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. "NAREL Standard Operating Procedure for Actinides in Environmental Matrices by Extraction Chromatography." AM/SOP-1. Revision 7. Montgomery, AL: U.S. EPA. For access to this procedure, consult the appropriate contact in Section 4.0.

6.2.27 EML HASL-300 Method Am-01-RC: Americium in Soil

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium in soil
Method Selected for: This method has been selected for confirmatory analysis of soil and sediment samples.

Description of Method: This method uses alpha spectrometry for determination of americium-241 in soil. Americium is leached from soil with nitric acid and hydrochloric acid. Americium-243 is added as a tracer to determine chemical yield. The soil is processed through the plutonium separation steps using ion exchange resin according to Method Pu-11-RC. Americium is collected with a calcium oxalate precipitation and finally isolated and purified by ion exchange. After source preparation by microprecipitation, americium-241 is determined by alpha spectrometry analysis. The counting period chosen depends on the sensitivity required of the measurement and the acceptable degree of uncertainty in the result. The lower limit of detection (LLD) for americium-241 is 0.5 milli Becquerel (mBq) when counted for 1,000 minutes. In cases where less than 100 g of sample is available, use of EML HASL-300 Method Pu-12-RC is recommended.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). High levels of ammonium compounds or polyacrylamides (which can degrade into ammonium) present in some decontamination agents can potentially cause interference and should be removed during sample preparation by heating the extracts in nitric acid for 1 to 1.5 hours.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-01-RC: Americium in Soil." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-01-rc.pdf

6.2.28 EML HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium (but not lanthanides) in water and air filters
Method Selected for: This method has been selected for confirmatory analysis of drinking water, aqueous/liquid-phase samples, surface wipes and air filters.

Description of Method: This method is specific to measurement of americium isotopes in samples that do not contain lanthanides, but also can be used for measurement of californium and curium. The method uses microprecipitation and determination by alpha spectrometry. Americium-243 is added to the sample to determine chemical yield. The sample is processed through separation steps using ion exchange resins. The eluate from the ion exchange column containing americium (and all other ions, except plutonium) is evaporated, redissolved, and loaded onto a TRU resin extraction column. Americium (and curium and californium, if present) is separated and purified on the column and finally stripped with dilute nitric acid stripping solution. Microprecipitation is used to prepare for alpha spectrometry. The LLD for total americium is 0.3 mBq when counted for 1,000 minutes.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). Lanthanides, if present, will not be removed by this method and will significantly reduce the resolution of the alpha spectrograph. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-04-RC:

Americium in QAP Water and Air Filters - Eichrom's TRU Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-04-rc.pdf

6.2.29 EML HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium and/or plutonium in vegetation
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Vegetation is either dry ashed in a ceramic crucible using a muffle furnace or wet ashed with nitric acid. Plutonium-236 and americium-243 tracers are added after dry ashing or before wet ashing. Wet ashing requires considerably more time and must be carefully monitored due to the highly reactive nature of vegetation. The sample is further digested with hydrofluoric acid to dissolve silicate compounds. Plutonium is separated by ion exchange and determined by alpha spectrometry using the plutonium-236 tracer to determine recovery. Americium (and californium-252 and curium-244, if present) is collected with calcium oxalate precipitation and finally isolated and purified by ion exchange. After source preparation by microprecipitation, americium-241 (and californium-252 and curium-244, if present) is determined by alpha spectrometry using the americium-243 tracer to provide recovery data.

Special Consideration: PTFE beakers must be used when digesting samples with hydrofluoric acid. Clays that are present in some decontamination agents can contain iron, magnesium or calcium, which can be released via ion exchange in the presence of certain radionuclides and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resins used in this method.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-06-rc.pdf

6.2.30 EML HASL-300 Method Ga-01-R: Gamma Radioassay

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Gallium-68	15757-14-9
Gamma	NA
Germanium-68	15756-77-1
Indium-111	15750-15-9
Iodine-131	10043-66-0
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Neptunium-239	13968-59-7
Rhenium-188	14378-26-8
Rubidium-82	14391-63-0
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA
Technetium-99m	378784-45-3

Analysis Purpose: Qualitative and confirmatory analysis or gross gamma determination
Technique: Gamma spectrometry

Method Developed for: Gamma-ray emitting radionuclides in a variety of environmental matrices
Method Selected for: This method has been selected for qualitative and/or confirmatory analysis of select gamma emitters in aqueous/liquid-phase, soil and sediment, surface wipes, air filters and/or vegetation.

Description of Method: This method uses gamma spectrometry for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. Samples are placed into a standard geometry for counting, typically using a high purity Germanium [HP(Ge)] detector. Ge(Li) or NaI(Tl) detectors also can be used. The sample is placed into a standard geometry for gamma counting. Soil samples and sludge are placed into an appropriately sized Marinelli beaker after drying and grinding for homogenization. Air filters and surface wipes can be counted directly or pressed into a planchet and counted. Samples are counted long enough to meet the required sensitivity. For typical counting systems and sample types, activity levels of approximately 40 Bq are measured, and sensitivities as low as 0.002 Bq can be achieved. Because of electronic limitations, count rates higher than 2,000 counts per second (cps) should be avoided. High activity samples can be diluted, reduced in size, or moved away from the detector (a limited distance) to reduce the count rate. The method is applicable for analysis of samples that contain radionuclides emitting gamma photons with energies above approximately 20 keV for germanium (Ge) (both HP(Ge) and GeLi) detectors and above 50 keV for NaI(Tl) detectors.

Special Considerations: Clays and hydrated alumina, which are present in some decontamination agents, can sequester cesium-137 and iodine-131, respectively. Each would be released only upon complete dissolution and, therefore, not measured when using this method for analysis of water samples. Compounds containing carbonate, fluoride, hydroxide or phosphate, also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis, resulting in a lesser amount of gamma emitting radionuclides in cases where a water sample aliquot is transferred and analyzed separately from the entire sample.

For qualitative analysis of the germanium-68 and gallium-68 pair, long count times may be required to meet detection limits as the 1077 KeV peak has a 3% abundance; for confirmatory analysis, the 511 KeV (176 abundance) should be larger than normal.

When detecting rubidium-82 (75 second half-life) by gamma spectroscopy in environmental samples, it is measured in equilibrium with its parent, strontium-82 (25.5 day half-life).

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ga-01-R: Gamma Radioassay." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-06/documents/eml-ga-01-r.pdf

6.2.31 EML HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters

Analyte(s)	CAS RN
Polonium-210	13981-52-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Polonium in water, vegetation, soil and air filters
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water, aqueous/liquid-phase, soil and sediment, and/or vegetation samples.

Description of Method: This method uses alpha spectrometry for determination of polonium in water, vegetation, soil and air filter samples. Polonium equilibrated with polonium-208 or polonium-209 tracer is isolated from most other elements by coprecipitation with lead sulfide. The sulfide precipitate is dissolved in weak hydrochloric acid solution. Polonium is quantitatively deposited on a nickel disc, and the plated disc is counted on an alpha spectrometer to measure chemical yield and activity of the sample. The solution from the deposition may be retained and analyzed for polonium-210. When counted for 1,000 minutes, the LLD for polonium is 1.0 mBq for water and 1.3 mBq for vegetation, soil and filters.

Special Considerations: This method requires specific valence states for radionuclides; oxidizing and reducing agents, which are present in decontamination agents, can impact the analysis. Oxidizers can oxidize nickel or lead to form soluble metal ions that can cause interferences. Chelating compounds, such as those present in some decontamination agents, can complex tightly to nickel and lead that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for polonium-210.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-07/documents/eml-po-02-rc.pdf

6.2.32 EML HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis

Technique: Alpha spectrometry

Method Developed for: Plutonium and americium in soil
Method Selected for: This method has been selected for use when small soil and sediment sample sizes (≤100 g) will be analyzed.

Description of Method: A sample of soil of up to 100 g is equilibrated with americium-243 tracer. Contaminant isotopes are leached with nitric and hydrochloric acid. Plutonium is removed by ion exchange. The eluent from the plutonium separation is saved for determination of americium, curium and californium. Americium, curium and californium are collected with a calcium oxalate coprecipitation, isolated and purified by extraction chromatography. Microprecipitation is used to prepare the sample for analysis by alpha spectrometry of americium, curium and californium. The LLD for americium is 0.5 mBq when counted for 1,000 minutes.

Special Considerations: In cases where only small sample sizes (≤100 g) will be analyzed, this method is recommended for confirmatory analysis. If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resins used in this method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium that can be released via ion exchange in the presence of certain radionuclides and cause analytical interferences.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-pu-12-rc.pdf

6.2.33 EML HASL-300 Method Ra-03-RC: Radium-226 in Soil, Vegetable Ash, and Ion Exchange Resin

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Radon emanation / Gamma spectroscopy (analysis of tracer)

Method Developed for: Radium-226 in soil, vegetation ash and ion exchange resin
Method Selected for: This method has been selected for qualitative and confirmatory analysis of vegetation.

Description of Method: Soil, vegetation ash or ion exchange resin are prepared for radon-222 emanation measurement. The sample is pretreated with nitric acid-hydrogen fluoride, fused with potassium fluoride and transposed to pyrosulfate. The cake is dissolved in dilute hydrochloric acid. Radium-barium sulfate is precipitated, filtered, and dissolved in alkaline EDTA. The chemical yield is determined with the γ-

emitting tracer barium-133. The solution is transferred to a radon bubbler. Radon is de-emanated into an ionization chamber or scintillation cell, and counted using a counter with a photomultiplier.

Special Consideration: Use of platinum crucibles is required in this method. Certain chelating compounds, which are present in some decontamination agents, can compromise the collection of radionuclides prior to analysis by preventing them from being precipitated out of solution during precipitation procedures. Other chelators can tightly complex barium or strontium that may be present in the sample, causing interference when analyzing for radium-226. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ra-03-RC: Radium 226 in Soil, Vegetable Ash, and Ion Exchange resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-ra-03-rc.pdf

6.2.34 EML HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Confirmatory analysis
Technique: Beta counting / Gamma spectroscopy (analysis of tracer)

Method Developed for: Strontium-90 in vegetation, water, air filters and soil
Method Selected for: This method has been selected for confirmatory analysis of soil and sediment samples, vegetation, surface wipes and air filters.

Description of Method: Strontium is separated from calcium, other fission products and natural radioactive elements. Fuming nitric acid separations remove the calcium and most other interfering ions. Radium, lead and barium are removed with barium chromate. Traces of other fission products are scavenged with iron hydroxide. After strontium-90 and yttrium-90 equilibrium has been attained, yttrium-90 is precipitated as the hydroxide and converted to oxalate for counting on a low-background gas proportional beta counter. Chemical yield is determined with strontium-85 tracer by counting in a gamma well detector.

Special Consideration: If analyzing highly calcareous soils, or if carbonate compounds found in some decontamination agents are present in the sample, an additional quantity of hydrochloric acid should be added to replace the acid required to decompose the carbonates. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-sr-03-rc.pdf

6.2.35 EML HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Confirmatory analysis
Technique: Beta counting / Gamma spectrometry

Method Developed for: Technetium-99 in water and vegetation
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Samples are wet ashed with nitric acid. After wet ashing is complete, samples are evaporated to the smallest volume possible with no salting out. The resulting solution is cooled, transferred to a 1-L beaker, and diluted to 800 mL with reagent water. The sample solution is then stirred and filtered with suction through a 15-cm glass fiber filter, and the filter is washed with water. The filter containing the silica and insoluble material is discarded. Technetium-99 is equilibrated with technetium-95m tracer in the wet ashing step. Technetium is separated from other elements by anion exchange and electro-deposition, and technetium-99 is beta counted. Gamma spectrometry measurement of technetium-95m tracer provides the chemical yield.

Special Consideration: Technetium-95m tracer is no longer readily available from the source cited in the method. If technetium-95m cannot be obtained, technetium-99m tracer may be substituted. Compounds containing carbonate, hydroxide, phosphate or sulfate, which are present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of technetium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-tc-01-rc.pdf

6.2.36 EML HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water samples.

Description of Method: The sample containing technetium-99 is mixed with technetium-95m added as a gamma-emitting tracer. The two isotopes of technetium are brought to an isotopic equilibrium and separated from other elements by ferrous and ferric hydroxide coprecipitation. The precipitate is dissolved with dilute nitric acid and passed through a TEVA-resin column, which is highly specific for technetium in the pertechnetate form. The resin is washed with dilute nitric acid to remove possible interferences and then it is eluted directly into a suitable liquid scintillation cocktail. The sample is typically counted for 1 hour to simultaneously determine technetium-99 activity and the technetium-95m radiochemical yield. Quench/efficiency calibration curves need to be established for the liquid scintillation spectrometer for both technetium-95m and technetium-99.

Special Considerations: Chemical and color quenching can have a significant impact when using liquid scintillation methods. Several compounds contained in decontamination agents can cause this quenching, such as organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds. Chelators can compromise the collection of radionuclides prior to analysis, by causing them to avoid being precipitated out of solution during precipitation procedures.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-tc-02-rc.pdf

6.2.37 EML HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry
Method Developed for: Isotopic uranium in biological and environmental materials
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Uranium from acid leached, dry-ashed and wet-ashed materials is equilibrated with uranium-232 tracer, and isolated by anion exchange chromatography. The separated uranium isotopes are microprecipitated for alpha spectrometry.

Special Considerations: For microprecipitation procedures, refer to HASL-300 Method G-03. Chelating or complexing compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on the ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-u-02-rc.pdf

6.2.38 DOE FRMAC Method Volume 2, Page 33: Gross Alpha and Beta in Air

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in air
Method Selected for: This method has been selected for gross alpha and gross beta determination in air filters, for direct counting of surface wipes, and for qualitative analysis of actinium-225 in surface wipes and air filters.

Description of Method: A gas-flow proportional counter is used for counting gross alpha and beta radioactivity. The method supplies an approximation of the alpha and beta activity present in the air or the removable surface activity dependent on the sample type. The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Actinium-225 (CAS RN 14265-85-1) Alpha emitter
- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter

- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Thorium-227 (CAS RN 15623-47-9) Alpha emitter
- Thorium-228 (CAS RN 14274-82-9) Alpha emitter
- Thorium-230 (CAS RN 14269-63-7) Alpha emitter
- Thorium-232 (CAS RN 17440-29-1) Alpha emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

For this application, the procedure requires the use of thorium-230 for alpha counting efficiency and cesium-137 for beta counting efficiency in the calibration of the detector. An air filter or swipe sample is placed onto a planchet, then counted for alpha and beta radioactivity. Activity is reported in activity units per volume of air sampled, as units of activity per surface area sampled, or as total units of activity in cases where sample collection information is not available.

Special Considerations: High levels of particulate loading on the air filter or swipe will affect the alpha efficiency. Accurate results for radionuclides, other than cesium-137 and thorium-230, may be difficult because of the difference in efficiencies for the uncalibrated radionuclides. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Gross alpha screening may be used for qualitative analysis of actinium-225. For every one actinium-225 decay, there are up to four alpha particles emitted depending on daughter equilibrium. To determine the qualitative result for actinium-225, the gross alpha result should be divided by four.

Source: FRMAC. 1998. "Gross Alpha and Beta in Air." *FRMAC Monitoring and Analysis Manual – Sample Preparation and Analysis* - Volume 2, DOE/NV/11718-181 Vol. 2, UC-707, p. 33. Las Vegas, NV: U.S. DOE. http://www.epa.gov/sites/production/files/2015-06/documents/frmac-vol2-pg33.pdf

6.2.39 DOE RESL Method P-2: P-32 Fish, Vegetation, Dry Ash, Ion Exchange

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Čerenkov counting with Liquid Scintillation

Method Developed for: Phosphorus-32 in fish and vegetation
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil, sediment, wipes, air filters and vegetation.

Description of Method: Samples up to 500 g are dry ashed at 550°C and dissolved in two portions of nitric acid. The sample is evaporated to half volume and transferred to a perchloric acid hood. Concentrated nitric acid and concentrated perchloric acid are added, and the sample is evaporated to

dryness. The residue is dissolved in hydrochloric acid and filtered through a glass fiber filter. Iron-55 is removed by precipitation with cupferron. The solution containing phosphate is purified by passing it through anion and cation columns to remove possible contaminants. The purified phosphate is precipitated as magnesium ammonium phosphate, filtered onto a glass fiber filter, and dried. The magnesium ammonium phosphate is dissolved in nitric acid and transferred to a counting vial. Phopsphorus-32 is assayed by counting the Čerenkov radiation with a liquid scintillation counter.

Special Considerations: Laboratories using this method must have a designated perchloric acid fume hood. This method was developed for analysis of fish and vegetation. Additional development and testing is necessary for application to soil, sediment, wipes and air filters. Phosphorus and iron carrier must be added to matrices that do not contain milligram quantities of both elements.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Several compounds contained in decontamination agents can cause this quenching, such as organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds. Chelators also can tightly complex calcium that may be present in the sample, causing interference when analyzing for phosphorus-32.

Source: RESL, DOE. 1977. "Method P-2: P-32 Fish, Vegetation, Dry Ash, Ion Exchange." *RESL Analytical Chemistry Branch Procedures Manual*, IDO-12096.
http://www.epa.gov/sites/production/files/2015-07/documents/resl-p-2.pdf

6.2.40 DOE SRS Actinides and Sr-89/90 in Soil Samples

Analyte(s)	CAS RN
Strontium-89	14158-27-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry and beta counting

Method Developed for: Actinides and strontium-89 and -90 in soil samples
Method Selected for: This method has been selected for qualitative analysis of strontium-89 in soil and sediment samples.

Description of Method: Radioactive tracers are added to samples prior to sample fusion at 600°C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid, and aluminum nitrate. A column separation using TEVA, TRU and DGA resins is applied to separate the actinides into four fractions: thorium, plutonium-neptunium, uranium and americium/curium. Plutonium-242 (or plutonium-236 if neptunium-237 is measured), thorium-229, americium-243 and uranium-232 are used as tracers to determine yield. Actinide tracers are not needed when analyzing samples only for Sr-89. The various fractions of actinides are eluted from the resin columns and precipitated with cerium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.

- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.
- Reducing agents also can impact this method, which requires specific valence states for radionuclides.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Soil." *SRS Manual L3.23*, Procedure L3.23-10054. http://www.epa.gov/sites/production/files/2015-07/documents/l3.23-10054.pdf

6.2.41 DOE SRS Actinides and Sr-89/90 in Vegetation: Fusion Method

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15517-48-3
Strontium-89	14158-27-1
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry / Beta counting

Method Developed for: Actinides and strontium-89 and -90 in vegetation
Method Selected for: This method has been selected for qualitative analysis of vegetation.

Description of Method: Radioactive tracers are added to samples prior to sample fusion at 600°C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid and aluminum nitrate. A column separation using commercially available resins (TEVA, TRU and DGA) is applied to separate the actinides into three fractions: plutonium/neptunium, uranium and americium/curium. Plutonium-242 (or plutonium-236 if neptunium-237 is measured), thorium-229, americium-243 and uranium-232 are used as tracers to determine yield. The various fractions of actinides are eluted from the resin columns and precipitated with cerium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: Thorium-228, if present as a daughter of uranium-232 tracer, will interfere with thorium-228 analysis. Self-cleaning uranium-232 tracer, with thorium-228 removed, is required if thorium isotopes are separated and measured with uranium. If uranium-232 is present in a sample, the procedure of standard addition can be used to determine the amount of uranium-232 contamination. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability

and/or poison extraction resins used in this method.
- Reducing agents also can impact this method, which requires specific valence states for radionuclides.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Vegetation: Fusion Method." *SRS Manual L3.23*, Procedure L3.23-10055. http://www.epa.gov/sites/production/files/2015-07/documents/l3.23-10055.pdf

6.2.42 ORISE Method AP1: Gross Alpha and Beta for Various Matrices

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in water, soil, vegetation and other solids
Method Selected for: This method has been selected for gross alpha and gross beta determination in soil, sediment, and vegetation samples and qualitative analysis of actinium-225 in soil, sediment and vegetation samples.

Description of Method: This method provides an indication of the presence of alpha and beta emitters, including the following analytes:

• Actinium-225	(CAS RN 14265-85-1)	Alpha emitter
• Americium-241	(CAS RN 14596-10-2)	Alpha emitter
• Californium-252	(CAS RN 13981-17-4)	Alpha emitter
• Cesium-137	(CAS RN 10045-97-3)	Beta emitter
• Cobalt-60	(CAS RN 10198-40-0)	Beta emitter
• Curium-244	(CAS RN 13981-15-2)	Alpha emitter
• Europium-154	(CAS RN 15585-10-1)	Beta emitter
• Iridium-192	(CAS RN 14694-69-0)	Beta emitter
• Plutonium-238	(CAS RN 13981-16-3)	Alpha emitter
• Plutonium-239	(CAS RN 15117-48-3)	Alpha emitter
• Polonium-210	(CAS RN 13981-52-7)	Alpha emitter
• Radium-226	(CAS RN 13982-63-3)	Alpha emitter
• Ruthenium-103	(CAS RN 13968-53-1)	Beta emitter
• Ruthenium-106	(CAS RN 13967-48-1)	Beta emitter
• Strontium-90	(CAS RN 10098-97-2)	Beta emitter
• Thorium-227	(CAS RN 15623-47-9)	Alpha emitter
• Thorium-228	(CAS RN 14274-82-9)	Alpha emitter
• Thorium-230	(CAS RN 14269-63-7)	Alpha emitter
• Thorium-232	(CAS RN 17440-29-1)	Alpha emitter
• Uranium-234	(CAS RN 13966-29-5)	Alpha emitter
• Uranium-235	(CAS RN 15117-96-1)	Alpha emitter
• Uranium-238	(CAS RN 7440-16-1)	Alpha emitter

This procedure provides screening measurements to indicate whether specific analyses are required for water, soil, vegetation and other solids. Liquid samples are acidified, concentrated, dried in a planchet, and counted in a low-background proportional counter. Solid samples are dried and homogenized, and a known quantity is transferred to a planchet and counted in a low-background proportional counter.

Special Considerations: Volatile radionuclides will not be accurately determined using this procedure. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Gross alpha screening may be used for qualitative analysis of actinium-225. For every one actinium-225

decay, there are up to four alpha particles emitted depending on daughter equilibrium. To determine the qualitative result for actinium-225, the gross alpha result should be divided by four.

Source: ORISE, Oak Ridge Associated Universities (ORAU). 2001. "Method AP1: Gross Alpha and Beta for Various Matrices." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap1.pdf

6.2.43 ORISE Method AP2: Determination of Tritium

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium in soil, sediment, animal tissue, vegetation, smears and water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil and sediment, surface wipes and vegetation.

Description of Method: The tritium in aqueous and solid samples is distilled using an Allihn condenser. For solid samples, an appropriate volume of laboratory reagent water is added to facilitate distillation. Certain solid samples may be refluxed to ensure distribution of any tritium that may be in the sample. The sample may be spiked with a standard tritium solution to evaluate quenching and counting efficiency. After the sample has been distilled, an aliquot of the distillate is added to a scintillation cocktail and the sample is counted using a liquid scintillation analyzer.

Special Considerations: Other volatile radionuclides such as iodine and carbon isotopes may interfere and may require that the sample be made alkaline using solid sodium hydroxide before distillation. Organic impurities may interfere and may require the addition of an oxidizing agent to the sample as well as spiking the samples with a standard tritium solution. The addition of a standard tritium solution to each sample allows for counting efficiencies to be calculated for each individual sample.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds that can cause chemical or color quenching.

Source: ORISE, ORAU. 2001. "Method AP2: Determination of Tritium." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*.
http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap2.pdf

6.2.44 ORISE Method AP5: Determination of Technetium-99

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in sediment, soil, smears and water at environmental levels
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil

and sediment, surface wipe and air filter samples; and qualitative analysis of vegetation.

Description of Method: Solid samples are leached with dilute nitric acid. The leachates are passed through a TEVA-resin column, which is highly specific for technetium in the pertechnetate form. The technetium is absorbed onto the extraction resin. The resin is added to a scintillation vial containing an appropriate cocktail and counted using a liquid scintillation analyzer. Most interfering beta emitting radionuclides (including carbon-14, phosphorus-32, sulfur-35, strontium-90, yttrium-90 and thorium-234) are effectively removed using TEVA resin under the conditions in this procedure.

Special Considerations: Tritium may follow technetium due to the absorption of some tritium-labeled compounds by the resin. Possible tritium interferences are eliminated by setting the technetium counting window above the maximum energy of tritium beta particles. Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis by preventing them from being precipitated out of solution during precipitation procedures. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds can cause chemical or color quenching.

Source: ORISE, ORAU. 2001. "Method AP5: Determination of Technetium-99." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap5.pdf

6.2.45 ORISE Method AP7: Determination of Radium-226 in Water and Soil Samples Using Alpha Spectroscopy

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry / Gamma spectroscopy (analysis of tracer)
Method Developed for: Radium-226 in water and soil
Method Selected for: This method has been selected for confirmatory analysis of radium-226 in soil and sediment samples.

Description of Method: The tracer (barium-133) and potassium hydrogen fluoride are added to a soil sample aliquot in a platinum crucible. The sample is heated until the potassium hydrogen fluoride has completely dried. Heating is continued at 900°C until total dissolution of the sample. After allowing the sample to cool slightly, sulfuric acid is added and the mixture is heated to dissolve the fluoride cake. Sodium sulfate is added to the slurry and the temperature is slowly raised until the slurry melts completely. Water and 12M hydrochloric acid are added to dissolve the pyrosulfate cake. Sulfuric acid (9M), potassium sulfate and sodium sulfate are added and the solution is evaporated to 35–40 mL. Three portions of lead (II) in solution are added while stirring, waiting 5 minutes between each addition. After centrifuging, the supernatant is discarded and the precipitate is dissolved in diethylenetriaminepentaacetic acid (DTPA). Barium (II) in solution is added to form barium sulfate, which acts to separate radium-226 from possible interfering radionuclides. The barium precipitate is filtered, and radium-226 is counted by alpha spectroscopy. Barium-133 is used to quantify the yield by gamma spectroscopy.

Special Considerations: High levels of barium will add mass to the final sample, causing self-attenuation and degradation of the alpha spectrum. If the amount of barium in the sample can be predetermined, it may be possible to adjust sample size and not add the barium (II) in step 4.2.11 of the method. Contamination with barium-133 will interfere with the yield determination. This may be corrected by gamma counting before analysis and adjusting the barium yield accordingly.

Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis by preventing them from being precipitated out of solution during precipitation procedures. Other chelators can tightly complex barium or strontium that may be present in the sample, causing interference when analyzing for radium-226. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well.

Source: ORISE, ORAU. 2001. "Method AP7: Determination of Radium-226 in Water and Soil Samples Using Alpha Spectroscopy." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*.
https://www.epa.gov/sites/production/files/2017-10/documents/ap7_ra-226_water_soil_alpha_spec.pdf

6.2.46 ORISE Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium, curium, plutonium, neptunium, thorium and/or uranium in water and solid samples
Method Selected for: This method has been selected for confirmatory analysis when a sample exists in a refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem. In the event of refractory radioactive material, this method is recommended for both qualitative determination and confirmatory analysis of drinking water, aqueous/liquid-phase, soil and sediment, surface wipes and air filter samples.

Description of Method: Solid and unfiltered aqueous samples (after evaporation to dryness) are dissolved completely by a combination of potassium hydrogen fluoride and pyrosulfate fusions. Filtered aqueous samples are evaporated to dryness followed by a pyrosulfate fusion. The fusion cake is dissolved and, for analyses requiring uranium only, two barium sulfate precipitations are performed, and the uranium is separated using EDTA. For all other analyses, one barium sulfate precipitation is performed and all alpha emitters are coprecipitated on barium sulfate. The barium sulfate is dissolved and the actinides are separated by extraction chromatography. An optional section is presented for the separation of americium from the lanthanides. All actinides are coprecipitated on cerium fluoride and counted with an alpha spectrometer system.

Special Considerations: Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium or calcium that can be released via ion exchange in the presence of

certain radionuclides and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: ORISE, ORAU. 2001. "Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap11.pdf

6.2.47 ORISE Method Procedure #9: Determination of I-125 in Environmental Samples

Analyte(s)	CAS RN
Iodine-125	14158-31-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Iodine-125 in environmental samples, such as soil, sediment, vegetation, water, milk, filters (air or water), etc.
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water, aqueous/liquid-phase, soil and sediment, surface wipe, air filter and vegetation samples.

Description of Method: In this method a direct comparison is made between the sample and a source prepared from a National Institute of Standards and Technology (NIST) traceable standard. If it is known, either by the sample preparation procedure or by a qualitative analysis on some device (high resolution intrinsic planar detector) that iodine-125 is the only radionuclide contributing to the observed peak, then this method can be used as a rapid quantitative method.

The sample is prepared by matrix specific techniques and the final sample is placed in a 16-mL culture tube and counted in a 3" x 3" sodium iodide (NaI(Tl)) well detector attached to a pulse height analyzer. Iodine-125 gamma counting rate is determined in the 25 to 35 keV energy range by pulse height analysis. NIST traceable liquid standards are also counted in the same geometric configuration as the samples to determine iodine-125 counting efficiency.

Special Considerations: Due to the low photon energy of iodine-125, the Compton scattering and x-ray photons from other radionuclides may cause significant interferences in this procedure. Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column. Hydrated alumina, also present in certain decontamination agents, can sequester iodine-125 and iodine-131, which would only be released upon complete dissolution and therefore not be measured when using this method.

Source: ORISE, ORAU. 1995. "Procedure #9: Determination of I-125 in Environmental Samples." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-procedure9-1995.pdf

6.2.48 ASTM Method D3084-20: Standard Practice for Alpha Spectrometry in Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Alpha particle spectra in water
Method Selected for: This method has been selected for qualitative determination of californium-252 and curium-244 in drinking water, surface wipes, air filters and vegetation; americium-241, californium-252, curium-244, and plutonium-238 and -239 in aqueous/liquid-phase samples; and californium-252 and curium-244 in soil and sediment.

Description of Method: This standard practice covers the process that is required to obtain well-resolved alpha spectra from water samples and discusses the associated problems. This practice is typically preceded with specific chemical separations and mounting techniques that are included in referenced methods. A chemical procedure is required to isolate and purify the radionuclides (see Section 10.1 of the method), and a radioactive tracer is added to determine yield. A source is prepared by employing electrodeposition, microprecipitation or evaporation (depositing the solution onto a stainless steel or platinum disc). Electrodeposition and microprecipitation are preferred. The source's radioactivity is then measured in an alpha spectrometer according to manufacturer's operating instructions. The counting period chosen depends on the sensitivity required of the measurement and the degree of uncertainty in the result that is acceptable.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46) for sample preparation instead of the methods referenced in ASTM Method D3084. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: ASTM. 2020. "Method D3084-20: Standard Practice for Alpha Spectrometry in Water." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D3084.htm

6.2.49 ASTM Method D3972-09 (2015): Standard Test Method for Isotopic Uranium in Water by Radiochemistry

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Alpha-particle-emitting isotopes of uranium in water
Method Selected for: This method has been selected for confirmatory analysis of drinking water samples.

Description of Method: Uranium is chemically separated from a water sample by coprecipitation with ferrous hydroxide followed by anion exchange, and electrodeposition. When suspended matter is present, an acid dissolution step is added to ensure that all of the uranium dissolves. The sample is acidified, and uranium-232 is added as an isotopic tracer to determine chemical yield. Uranium is coprecipitated from the sample with ferrous hydroxide. This precipitate is dissolved in concentrated hydrochloric acid, or is subjected to acid dissolution with concentrated nitric and hydrofluoric acids, if the hydrochloric acid fails to dissolve the precipitate. Uranium is separated from other radionuclides by adsorption on anion exchange resin, followed by elution with hydrochloric acid. The uranium is finally electrodeposited onto a stainless steel disc and counted using alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, hydroxide, or phosphate can precipitate uranium out of solution prior to analysis.

Source: ASTM. 2015. "Method D3972-09 (2015): Standard Test Method for Isotopic Uranium in Water by Radiochemistry." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D3972.htm

6.2.50 ASTM Method D5811-20: Standard Test Method for Strontium-90 in Water

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid-phase samples.

Description of Method: An aliquot of the sample is measured into a beaker, and strontium carrier is added. The sample is digested with nitric acid, sorbed on an ion exchange column, eluted, and evaporated to dryness. The residue is redissolved in nitric acid and then is selectively sorbed on a solid phase extraction column. Strontium is eluted with dilute nitric acid, dried on a planchet, weighed, and counted for beta radiation.

Special Considerations: Significant amounts of stable strontium, if present in the sample, will interfere with the yield determination. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed

separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, fluoride, phosphate or sulfate can precipitate radionuclides out of solution prior to analysis, resulting in a lesser amount of strontium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: ASTM. 2020. "Method D5811-20: Standard Test Method for Strontium-90 in Water." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D5811.htm

6.2.51 ASTM Method D7168-16: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid-phase samples.

Description of Method: A measured aliquot of sample is transferred to a beaker and hydrogen peroxide is added to facilitate the formation of the extractable pertechnetate ion. The sample may be heated to oxidize organics, if suspected to be present. The entire sample is passed through a technetium-selective solid-phase extraction (SPE) disk onto which the pertechnetate is adsorbed. The disk is transferred to a liquid scintillation vial, scintillation cocktail is added, and the contents are well mixed. The beta-emission rate of the sample is determined by liquid scintillation spectrometry. Chemical yield corrections are determined by the method of standard additions.

Special Considerations: Suspended materials must be removed by filtration or centrifuging prior to processing the sample. High levels of iodate, iron (III) and antimony can interfere with the measurement of technetium-99 and lead to a positive bias in sample results.

Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being precipitated out of solution during precipitation procedures. Chemical and color quenching can have a significant impact when using liquid scintillation methods. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds that can cause chemical or color quenching, which can significantly impact liquid scintillation methods.

Source: ASTM. 2016. "Method D7168-16: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7168.htm

6.2.52 Standard Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta activity in water
Method Selected for: This method has been selected for gross alpha and gross beta determination in aqueous/liquid-phase samples and qualitative analysis of actinium-225 in aqueous/liquid-phase samples.

Description of Method: This method allows for measurement of gross alpha and gross beta radiation in water samples. The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

• Actinium-225	(CAS RN 14265-85-1)	Alpha emitter
• Americium-241	(CAS RN 14596-10-2)	Alpha emitter
• Californium-252	(CAS RN 13981-17-4)	Alpha emitter
• Cesium-137	(CAS RN 10045-97-3)	Beta emitter
• Cobalt-60	(CAS RN 10198-40-0)	Beta emitter
• Curium-244	(CAS RN 13981-15-2)	Alpha emitter
• Europium-154	(CAS RN 15585-10-1)	Beta emitter
• Iridium-192	(CAS RN 14694-69-0)	Beta emitter
• Plutonium-238	(CAS RN 13981-16-3)	Alpha emitter
• Plutonium-239	(CAS RN 15117-48-3)	Alpha emitter
• Polonium-210	(CAS RN 13981-52-7)	Alpha emitter
• Radium-226	(CAS RN 13982-63-3)	Alpha emitter
• Ruthenium-103	(CAS RN 13968-53-1)	Beta emitter
• Ruthenium-106	(CAS RN 13967-48-1)	Beta emitter
• Strontium-90	(CAS RN 10098-97-2)	Beta emitter
• Thorium-227	(CAS RN 15623-47-9)	Alpha emitter
• Thorium-228	(CAS RN 14274-82-9)	Alpha emitter
• Thorium-230	(CAS RN 14269-63-7)	Alpha emitter
• Thorium-232	(CAS RN 17440-29-1)	Alpha emitter
• Uranium-234	(CAS RN 13966-29-5)	Alpha emitter
• Uranium-235	(CAS RN 15117-96-1)	Alpha emitter
• Uranium-238	(CAS RN 7440-16-1)	Alpha emitter

This method recommends using a thin-window gas-flow proportional counter for counting gross alpha and beta radioactivity. An internal proportional or Geiger counter may also be used. An aliquot of sample is evaporated to a small volume and transferred to a tared counting pan. The sample residue is dried to constant weight, cooled, and reweighed to determine dry residue weight, then counted for alpha and beta radioactivity.

Special Considerations: Ground water samples containing elevated levels of dissolved solids will require use of smaller sample volumes. Compounds containing carbonate, fluoride, hydroxide, or phosphate, which are present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of radionuclides in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. The presence of hydroxide, in particular, can have a significant impact. At a pH above 5, it will precipitate out iron, carrying actinides (e.g., uranium and plutonium) with it.

Gross alpha screening may be used for qualitative analysis of actinium-225. For every one actinium-225 decay, there are up to four alpha particles emitted depending on daughter equilibrium. To determine the qualitative result for actinium-225, the gross alpha result should be divided by four.

Source: APHA, AWWA and WEF. 2017. "Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.53 Standard Method 7120: Gamma-Emitting Radionuclides

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Iridium-192	14694-69-0
Neptunium-239	13968-59-7
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5

Analysis Purpose: Qualitative and confirmatory determination
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of select gamma emitters in aqueous/liquid-phase samples.

Description of Method: The method uses gamma spectroscopy using either Ge detectors or NaI(Tl) crystals for the measurement of gamma photons emitted from radionuclides present in water. The method can be used for qualitative and confirmatory determinations with Ge detectors or semi-qualitative and semi-quantitative determinations (using NaI(Tl) detectors). Exact confirmation using NaI is possible for single nuclides or when the gamma emissions are limited to a few well-separated energies. A homogeneous water sample is placed into a standard geometry (normally a Marinelli beaker) for gamma counting. Sample portions are counted long enough to meet the required sensitivity of measurement. A radioactive standard, in the same geometry as the samples, containing a mixture of gamma energies from approximately 50 to 2000 keV is used for energy and efficiency calibration.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Reducing agents can potentially convert radionuclides into an insoluble zero-valent state that can precipitate out of solution. The addition of nitric acid during sample collection can prevent this precipitation from occurring. Iridium and ruthenium would likely still precipitate in the presence of reducing agents.
- Clays can sequester cesium-137, which would only be released upon complete dissolution when using this method.
- Compounds containing carbonate, fluoride, hydroxide, or phosphate can precipitate radionuclides out of solution prior to analysis.

Source: APHA, AWWA and WEF. 2017. "Method 7120: Gamma-Emitting Radionuclides." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.54 Standard Method 7500-Ra B: Radium: Precipitation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative analysis
Technique: Alpha counting

Method Developed for: Alpha-emitting isotopes of radium in water
Method Selected for: This method has been selected for qualitative determination in aqueous/liquid-phase samples.

Description of Method: This method is for determination of all alpha-emitting radium isotopes by alpha decay analysis. Lead and barium carriers are added to the sample containing alkaline citrate, then sulfuric acid is added to precipitate radium, barium and lead as sulfates. The precipitate is purified by washing with nitric acid, dissolving in alkaline EDTA, and re-precipitating as radium-barium sulfate after pH adjustment to 4.5. This slightly acidic EDTA keeps other naturally occurring alpha-emitters and the lead carrier in solution. Radium-223, -224 and -226 are identified by the rate of ingrowth of their daughter products in barium sulfate precipitate. The results are corrected by the rate of ingrowth of daughter products to determine radium activity. This method involves alpha counting by a gas-flow internal proportional counter, scintillation counter or thin end-window gas-flow proportional counter.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds, such as those contained in some decontamination agents, can complex tightly to calcium, nickel, lead, magnesium or strontium that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for radium-226.
- Compounds containing sulfate, carbonate, oxalate or phosphate, which are also present in some decontamination agents, can precipitate radium out of solution prior to analysis.

Source: APHA, AWWA and WEF. 2017. "Method 7500-Ra B: Radium: Precipitation Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.55 Standard Method 7500-Ra C: Radium: Emanation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha counting

Method Developed for: Soluble, suspended and total radium-226 in water
Method Selected for: This method has been selected for confirmatory analysis of aqueous/liquid-phase samples.
Description of Method: Radium in water is concentrated and separated from sample solids by coprecipitation with a relatively large amount of barium as the sulfate. The precipitate is treated to remove silicates, if present, and to decompose insoluble radium compounds, fumed with phosphoric acid

to remove sulfite, and dissolved in hydrochloric acid. The completely dissolved radium is placed in a bubbler, which is then closed and stored for a period of several days to 4 weeks for ingrowth of radon. The bubbler is connected to an evacuation system and the radon gas is removed from the liquid by aeration and helium, dried with a desiccant, and collected in a counting cell. Four hours after radon collection, the cell is counted. The activity of the radon is equal to the radium concentration. The MDC depends on counter characteristics, background-counting rate of scintillation cell, cell efficiency, length of the counting period, and contamination of the apparatus and environment by radium-226. Without reagent purification, the overall reagent blank (excluding background) should be between 0.03 and 0.05 pCi radium-226, which may be considered the minimum detectable amount under routine conditions.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation, Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can complex tightly to calcium, nickel, lead, magnesium or strontium that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for radium-226.
- Compounds containing sulfate, carbonate, oxalate or phosphate can precipitate radium out of solution prior to analysis. This precipitation can result in a lesser amount of radium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.
- Oxidizers present in these agents can oxidize nickel or lead to form soluble metal ions that can also cause interferences.

Source: APHA, AWWA and WEF. 2017. "Method 7500-Ra C: Radium: Emanation Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.56 Standard Method 7500-U B: Uranium: Radiochemical Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha counting

Method Developed for: Total uranium alpha activity in water
Method Selected for: This method has been selected for qualitative determination in aqueous/liquid-phase samples.

Description of Method: The sample is acidified with hydrochloric or nitric acid and boiled to eliminate carbonate and bicarbonate ions. Uranium is coprecipitated with ferric hydroxide and subsequently separated. The ferric hydroxide is dissolved, passed through an anion-exchange column, washed with acid, and the uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, the residual salt is converted to nitrate, and the alpha activity is counted by a gas-flow proportional counter or alpha scintillation counter.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). The presence of compounds contained in various decontamination

agents can impact the results of analysis using this procedure due to precipitation, Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Certain chelating agents, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures.
- Compounds containing carbonate, hydroxide, or phosphate, which are present in some decontamination agents, can precipitate uranium out of solution.

Source: APHA, AWWA and WEF. 2017. "Method 7500-U B: Uranium: Radiochemical Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.57 Standard Method 7500-U C: Uranium: Isotopic Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Isotopic content of the uranium alpha activity; determining the differences among naturally occurring, depleted and enriched uranium in water
Method Selected for: This method has been selected for confirmatory analysis of aqueous/liquid-phase samples.

Description of Method: This method is a radiochemical procedure for determination of the isotopic content of uranium alpha activity. The sample is acidified with hydrochloric or nitric acid and uranium-232 is added as an isotopic tracer. Uranium is coprecipitated with ferric hydroxide and subsequently separated from the sample. The ferric hydroxide precipitate is dissolved and the solution passed through an anion-exchange column. The uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, and the residual salt is converted to nitrate and electrodeposited onto a stainless steel disc and counted by alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.46). Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well. Compounds containing carbonate, hydroxide or phosphate, which are also present in some decontamination agents, can precipitate uranium out of solution prior to analysis, resulting in a lesser amount of uranium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: APHA, AWWA and WEF. 2017. "Method 7500-U C: Uranium: Isotopic Method." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.58 Y-12 (DOE) Preparation of Samples for Total Activity Screening

Analyte(s)	CAS RN
Total Activity Screening	NA

Analysis Purpose: Total activity screening
Technique: Liquid scintillation
Method Developed for: Total activity screening
Method Selected for: This method has been selected for gross total activity screening of drinking water, aqueous/liquid-phase, soil and sediment, wipe, air filter and vegetation samples.

Description of Method: Aqueous sample aliquots that require no preparation are added directly to the scintillation cocktail. Solid and semi-solid sample aliquots are digested in nitric acid on a hot plate, cooled, filtered, and diluted to a specified volume. Oil sample aliquots are weighed directly into a tared counting vial. A specified volume of liquid scintillation cocktail is added to each vial and mixed with the sample aliquot. The samples are then counted for total activity.

Special Considerations: The method assumes 100% counting efficiencies for both beta and alpha emitters. Low energy beta emitters will not be counted at 100% efficiency, which can introduce a negative bias in the measurement.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds present in certain decontamination agents, can cause quenching. Chelators, also present in decontamination agents, can tightly complex calcium that may be present in a sample, causing analytical interference (e.g., excessive levels of calcium can interfere with detection and measurement of phosphorus-32).

Source: Y-12 (DOE). 2005. "Preparation of Samples for Total Activity Screening." Procedure Y50-AC-65-7230. http://www.epa.gov/sites/production/files/2015-07/documents/y50-ac-65-7230.pdf

6.2.59 Georgia Institute for Technology: Method for the Determination of Radium-228 and Radium-226 in Drinking Water by Gamma-ray Spectrometry Using HPGE or Ge(Li) Detectors

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Radium-228 and radium-226 in drinking water
Method Selected for: This method has been selected for confirmatory analysis of drinking water samples for radium-226.

Description of Method: This method describes the measurement of radium-226 and radium-228 in finished drinking water samples and can be used to measure radium-226 and radium-228 separately. An aliquant of the sample is poured into a borosilicate beaker and a solution of barium chloride is added as a carrier. The sample aliquant is then stirred and heated to boiling. Concentrated sulfuric acid is added to the heated sample and radium is collected by coprecipitating it as a sulfate. The precipitate is collected on

preweighed filter paper, dried, and the filter paper reweighed to obtain a net weight of precipitate and assess the chemical efficiency of the coprecipitation. The filter paper with the precipitate is placed into containers appropriate for the gamma-ray detector being used. For measurement of radium-226, the sample is counted with a gamma-ray spectrometry system after the appropriate ingrowth period of radium progeny to reach the required detection limit (see Table 17.3 of the source method). The minimum detectable level for this method is 1.0 pCi/L.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Permanganate and permanganic acid can be reduced to insoluble manganese (IV) oxide, which can remove radium.
- Certain chelating agents may compromise the collection of radionuclides prior to analysis, by preventing them from being precipitated out of solution. Dispersants and corrosion inhibitors can have this chelating ability as well.
- Clays contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interference in the analysis of the water.
- Compounds containing sulfate, carbonate, oxalate, or phosphate can precipitate radium out of solution prior to analysis, resulting in a lesser amount of radium in cases where an aliquot of water sample is transferred prior to the precipitation step, and analyzed separately from the entire sample.

Excess barium and strontium in the drinking water sample can result in high chemical yields, sometimes exceeding 100 percent recovery. Since their concentrations are restricted in finished drinking water to low levels, the related bias would only be a concern if this method is used to measure source or waste waters. Additional information regarding potential interferences is provided in Section 4 of the method.

Source: Georgia Institute for Technology, Environmental Resource Center. December 2004. "Method for the Determination of Radium-228 and Radium-226 in Drinking Water by Gamma-ray Spectrometry Using HPGE or Ge(Li) Detectors*,"* Revision 1.2. Atlanta, GA: Georgia Institute for Technology. https://www.regulations.gov/document/EPA-HQ-OW-2018-0558-0048

6.2.60 Eichrom: Determination of ^{225}Ac in Water Samples

Analyte(s)	CAS RN
Actinium-225	14265-85-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry or gamma spectrometry

Method Developed for: Actinium-225 in water
Method Selected for: This method has been selected for confirmatory analysis of drinking water and aqueous/liquid-phase samples.

Description of Method: Actinium-225 is preconcentrated from water samples (up to 1 L) using a ferric hydroxide precipitation. After dissolution in hydrochloric acid, actinium-225 is separated using TRU and DGA-resin cartridges. Actinium-225 is then prepared for measurement using lanthanum fluoride or cerium fluoride microprecipitation onto Resolve Filters. Chemical recovery of actinium can be traced using actinium-227 (alpha spectrometry). Actinium-225 can be measured by alpha spectrometry (5.54 –

5.83 MeV) or gamma spectrometry (via its francium-221 daughter, 218 KeV, 11.44%).

Special Considerations: The alpha emission from the actinium-227 tracer only occurs in 1.38% of decays and use of actinium-227 tracer may be more efficient by measuring its thorium-227 or radium-223 daughters after a period of ingrowth and decay. For alpha spectrometry, the mass of lanthanum that can be added to use a yield tracer must be minimized (55 µg) to prevent degradation of the alpha spectra through self-absorption.

If samples are analyzed by gamma spectrometry using the francium-221 daughter, francium-221 should be in equilibrium with actinium-225 in less than one hour. Francium-221 has a 218 keV gamma ray with 11.44% abundance. With gamma spectroscopy detection, the lanthanum carrier is not limited to trace amounts and the yield of stable lanthanum can be determined by ICP-MS or ICP-AES.

Chelating agents, which are present in some decontamination agents, will interfere to varying extents by totally or partially complexing actinide elements. Dispersants and corrosion inhibitors, also present in decontamination agents, can have chelating ability as well. When chelating agents are present, alternate methods, such as coprecipitation from acid solutions (Section 6.2.26), should be considered. Clays that are present in some decontamination agents can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interferences.

Source: Eichrom Technologies, LLC. "Determination of ^{225}Ac in Water Samples." AN-2101. Lisle, IL: Eichrom Technologies, LLC. https://www.eichrom.com/wp-content/uploads/2021/10/AN-2101_Ac-225-in-Water-Samples.pdf

6.2.61 Eichrom: Determination of ^{225}Ac in Geological Samples

Analyte(s)	CAS RN
Actinium-225	14265-85-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry or gamma spectrometry

Method Developed for: Actinium-225 in geological samples
Method Selected for: This method has been selected for confirmatory analysis of soil and sediment, surface wipes, air filters and vegetation samples.

Description of Method: Soil or rock samples are pulverized to <1mm and dissolved by acid digestion or sodium hydroxide fusion. Actinium-225 is separated from the matrix using a ferric hydroxide precipitation. After dissolution in hydrochloric acid, actinium-225 is separated using TRU and DGA-resin cartridges. Actinium-225 is then prepared for measurement using cerium fluoride or lanthanum fluoride microprecipitation onto Resolve Filters. Chemical recovery of actinium can be traced using actinium-227 (alpha spectrometry). Actinium-225 can be measured by alpha spectrometry (5.54-5.83 MeV) or gamma spectrometry (via its francium-221 daughter, 218 KeV, 11.44%).

Special Considerations: This method was developed for analysis of soil and rock. Additional development and testing is necessary for application to surface wipes, air filters and vegetation samples. When using acid digestion for sample preparation, care must be taken to ensure dissolution of all silicates in large volume vegetation samples and glass fiber filters (sodium hydroxide fusion should be adequate).

The alpha emission from the actinium-227 tracer only occurs in 1.38% of decays and use of actinium-227 tracer may be more efficient by measuring its thorium-227 or radium-223 daughters after a period of ingrowth and decay. Some environmental samples (e.g., soil) can also contain lanthanides. For alpha

spectrometry, the mass of lanthanum that can be added to use a yield tracer must be minimized (55 µg) to prevent degradation of the alpha spectra through self-absorption. If recoveries are determined by ICP-MS or ICP-AES, initial levels of lanthanides in the sample may need to be determined.

If samples are analyzed by gamma spectrometry using the francium-221 daughter, the francium-221 should be in equilibrium with actinium-225 in less than one hour. Francium-221 has a 218 keV gamma ray with 11.44% abundance. With gamma spectroscopy detection, the lanthanum carrier is not limited to trace amounts and the yield of stable lanthanum can be determined by ICP-MS or ICP-AES.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium and calcium that can be released as ions via ion exchange in the presence of certain radionuclides and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: Eichrom Technologies, LLC. "Determination of ^{225}Ac in Geological Samples." AN-2102. Lisle, IL: Eichrom Technologies, LLC. https://www.eichrom.com/wp-content/uploads/2021/10/AN-2102_Ac-225-in-Geological-Samples.pdf

6.3 Method Summaries (Outdoor Infrastructure and Building Material Samples)

Summaries corresponding to the methods selected for analysis of outdoor infrastructure and building material samples listed in Appendix B2 are provided in Sections 6.3.1 through 6.3.9. These summaries contain information that has been extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix B2 to which the method applies, a brief description of the analytical method, and a link to the full version of the method or a source for obtaining a full version of the method. Summaries are provided for informational use. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

6.3.1 Rapid Radiochemical Method for Total Radiostrontium (Sr-90) In Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-89 and -90 in building materials
Method Selected for: This method has been selected for confirmatory analysis of strontium-90 in asphalt singles, asphalt paving materials, concrete, brick and limestone.

Description of Method: Strontium is solubilized and purified by sodium hydroxide fusion using procedures described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples, Section 6.3.8 for asphalt shingles and Section 6.3.9 for limestone samples, and purified from potentially interfering radionuclides and matrix constituents using a strontium-specific, rapid chemical separation method. The sample is equilibrated with strontium carrier, and preconcentrated by strontium/calcium carbonate coprecipitation from the alkaline fusion matrix. The carbonate precipitate is dissolved in hydrochloric acid and strontium is precipitated with calcium fluoride to remove silicates. The strontium fluoride precipitate is dissolved in strong nitric acid and the solution is passed through a Sr Resin extraction chromatography column. The sample test source is promptly counted on a gas flow proportional counter to determine the beta emission rate, which is used to calculate the total radiostrontium activity. The method is capable of satisfying a method uncertainty for total strontium-90 of 0.31 pCi/g at an analytical action level of 2.4 pCi/g, using a sample weight of 1.5 g and a count time of approximately 1.5 hours.

If differentiating between strontium-89 and strontium-90 is needed, then the same prepared sample can be recounted after ~10 days. If the initial and second counts agree (based on the expected ingrowth of yttrium-90) then strontium-89 is not present in significant amounts relative to strontium-90. Computational methods are available for resolving the concentration of strontium-89 and strontium-90 from two sequential counts of the sample (see Appendix B of the method). If significant amounts of strontium-89 are suspected, it can be determined more rapidly using Čerenkov counting; however, the minimum detectable activity levels will be higher than that of determination with gas proportional counting and may or may not meet measurement quality objectives.

Special Considerations: Count results should be monitored for detectable alpha activity and appropriate corrective actions should be taken when this is observed. Failure to address the presence of alpha emitters

in the sample test source may lead to high bias in the results, due to alpha-to-beta crosstalk.

Elevated levels of tetravalent plutonium, neptunium, cerium or ruthenium in the sample may hold up on the column and co-elute with strontium. The method uses an oxalic acid rinse that should address low to moderate levels of these interferences. Significant levels of strontium-90 also will interfere with the total radiostrontium analysis (see Appendix B of the method for an alternative approach should this situation arise). High levels of lead-210 can interfere with low level strontium analysis due to ingrowth of short-lived bismuth-210 during chemical separations, where lead is retained by the resin, but is not eluted. If lead-210 is known to be present in samples, minimizing the time between the final rinse and the elution of strontium to less than 15 minutes will minimize the levels of interfering bismuth-210.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Clays and other compounds containing iron, magnesium and calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, can cause interferences.
- High levels of iron or magnesium can impact exchange site availability and/or poison the extraction resins.
- Chelating compounds can tightly complex barium, iron, lead, magnesium and potassium, causing interference when analyzing for strontium-89 or -90.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Total Radiostrontium (Sr-90) In Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.2 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in building materials
Method Selected for: This method has been selected for confirmatory analysis of radium-226 in asphalt shingle, asphalt paving materials, concrete, brick and limestone.

Description of Method: A known quantity of radium-225 is used as the yield tracer in this analysis. The sample is fused using the procedures described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples, Section 6.3.8 for asphalt roofing matrix samples and Section 6.3.9 for limestone samples. Radium isotopes are removed from the fusion matrix using a carbonate precipitation step. The sample is acidified and loaded onto a cation exchange resin to remove interferences, such as calcium. Radium is eluted from the cation resin with 8M nitric acid. After evaporation of the eluate, the sample is dissolved in a minimal amount of 3M nitric acid and passed through Sr Resin to remove any barium present. This solution is then evaporated to dryness, redissolved in 0.02M hydrochloric acid, and passed through Ln Resin to remove interferences such as residual calcium, and to remove the initial actinium-225 present. The radium (including radium-226) is prepared for counting by microprecipitation with barium sulfate. The activity measured in the radium-226 region of interest is corrected for chemical yield based on the observed activity of the alpha peak at 7.07 MeV

(astatine-217, the third progeny of radium-225).

This method is suited for low-level measurements for radium-226 using alpha spectrometry and is capable of satisfying a method uncertainty of 0.83 pCi/g at an analytical action level of 6.41 pCi/g, using a sample aliquant of approximately 1 g and count time of 8 hours (or longer).

Special Considerations: Depending on actual spectral resolution, method performance may be compromised if samples contain high levels of other radium isotopes (e.g., ~3 times the radium-226 activity concentration) due to ingrowth of interfering decay progeny. Calcium, iron (+3 oxidation state), and radionuclides with overlapping alpha energies, such as thorium-229, uranium-234, and neptunium-237, will interfere if they are not removed effectively. Delaying the count significantly longer than one day may introduce positive bias in results near the detection threshold due to the decay progeny from the radium 225 tracer. If radium-226 measurements close to detection levels are required and sample counting cannot be performed within ~36 hours of tracer addition, the impact of tracer progeny tailing into the radium-226 may be minimized by reducing the amount of the tracer that is added to the sample. This will aid in improving the signal-to-noise ratio for the radium-226 peak by minimizing the amount of tailing from higher energy alphas of the radium-225 progeny. If actinium-225 is present prior to the final separation time and the flow rate through the column is too fast (>1.5 drops/second), then actinium-225 will break through the resin, resulting in a high bias in the tracer yield. Additional information regarding procedures to remove or minimize interferences is provided in Section 4.0 of the method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides and cause interferences.
- High levels of iron, manganese, calcium or magnesium might have an impact on exchange site availability and/or poisoning of the extraction resins used.
- Chelators can tightly complex barium, calcium, iron and magnesium that may be present in the sample, causing interferences when analyzing for radium-226.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-002. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.3 Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion

Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in concrete and brick samples.

Method Selected for: This method has been selected for preparation of concrete and brick samples to be analyzed for americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238.

Description of Method: Concrete and brick samples may be received as core samples, pieces of various sizes, dust or particles (wet or dry) from scabbling, or powder. The method is based on the rapid fusion, in zirconium crucibles, of a representative, finely ground (5–100 mesh sized) 1–1.5-gram aliquant using rapid sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a carbonate precipitation, followed by calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. These sample preparation procedures are performed prior to the chemical separation procedures described in the following:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: In samples where native constituents may be present that could interfere with determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of the native constituents in advance of chemical separation (using a separate aliquant of fused material) and make appropriate adjustments to the yield calculations or amount of carrier added. Concrete and brick can contain native barium or radium, which can cause interferences with the analysis of radium-226. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R-14-004. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.4 Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Uranium-234, -235 and -238 in concrete and brick samples
Method Selected for: This method has been selected for confirmatory analysis of uranium-234, uranium-235 and uranium-238 in asphalt shingles, asphalt building materials, concrete, brick and limestone.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify uranium isotopes by removing interfering radionuclides as well as other components of the sample matrix in order to prepare the uranium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Uranium-232 tracer, added to the building materials sample, is used as a yield monitor. A 1.0- to 1.5-gram sample is fused using the procedure described in Section 6.3.3 for concrete and brick samples, Section 6.3.7 for asphalt samples, Section 6.3.8 for asphalt roofing materials and Section 6.3.9 for limestone samples. The uranium isotopes are then removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. The sample test source is prepared by microprecipitation with cerium (III) fluoride. The method is capable of achieving a method uncertainty for uranium-234, uranium-235, and uranium-238 of 1.9 pCi/g at an analytical level of 14.7 pCi/g, using a sample weight of approximately 1 g and count time of at least 3 to 4 hours.

Special Considerations: Alpha-emitting radionuclides with peaks at energies that cannot be adequately resolved from the tracer or analyte (e.g., polonium-210 [5.304 MeV], thorium-228 [5.423 MeV, 5.340 MeV] and americium-243 [5.275 MeV, 5.233 MeV]) must be chemically separated to enable radionuclide-specific measurements (see Section 4.0 of the method for procedures to remove specific interferences). Non-radiological anions such as fluoride and phosphate that complex uranium ions may cause lower chemical yields. Aluminum that is added in the column load solution complexes fluoride, as well as any residual phosphate that may be present. Lanthanum, added to preconcentrate uranium from the sample matrix as lanthanum fluoride, can have a slight adverse impact on uranium retention on TRU resin, but this impact is minimal at the level added. Iron (3+) can also have an adverse impact on uranium retention on TRU resin, but the residual iron levels after preconcentration steps are acceptable.

Clays, which are present in some decontamination agents, can contain iron, magnesium or calcium that can be released as ions via ion exchange in the presence of certain radionuclides and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison extraction resins used in alpha spectrometry. Higher valence anions such as phosphates may lead to lower yields when using the evaporation option due to competition with active sites on the resin. Concrete and brick can contain native uranium isotopes, which can cause interferences with the analysis of uranium isotopes.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-005. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.5 Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in building materials
Method Selected for: This method has been selected for confirmatory analysis of plutonium-238 and -239 in asphalt shingles, asphalt paving materials, concrete, brick and limestone.

Description of Method: This method is based on the use of TEVA resin to isolate and purify plutonium by removing interfering radionuclides as well as other components of the sample matrix in order to prepare the plutonium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. The sample may be fused using the procedure described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples, Section 6.3.8 for asphalt roofing matrix samples and Section 6.3.9 for limestone samples. The plutonium isotopes are then removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. Plutonium-242 or plutonium-236 tracer, added to the sample, is used as a yield monitor. The sample test source is prepared by microprecipitation with cerium (III) fluoride. The method is capable of achieving a required method uncertainty of 0.25 pCi/g for plutonium-238, -239/240, at an analytical action level of 1.89 pCi/g, using a sample weight of approximately 1 g and a count time of at least 3 to 4 hours.

Special Considerations: Alpha-emitting radionuclides with irresolvable alpha energies, such as plutonium-238 (5.50 MeV), americium-241 (5.48 MeV) and thorium-228 (5.42 MeV) must be chemically separated to enable measurement. This method separates these radionuclides effectively. The significance of peak overlap is determined by the detector's alpha energy resolution characteristics and the quality of the final precipitate that is counted.

Non-radiological interferences include very high levels of anions such as phosphates, which may lead to lower yields due to competition with active sites on the resin and/or complexation with plutonium ions. The presence of fluoride (e.g., from hydrofluoric or fluoroboric acids) can precipitate out plutonium prior to sample measurement. Aluminum is added in the column load solution to complex interfering anions such as fluoride and phosphate. Compounds such as clays containing iron, magnesium or calcium, which are present in some decontamination agents, can release these elements as ions via ion exchange in the presence of certain radionuclides and cause interference. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison extraction resins used in alpha spectrometry.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-006. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.6 Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241 in building materials
Method Selected for: This method has been selected for confirmatory analysis of americium-241 in asphalt shingles, asphalt paving materials, concrete, brick and limestone.

Description of Method: This method is based on the use of extraction chromatography resins (TEVA and DGA resins) to isolate and purify americium by removing interfering radionuclides as well as other matrix components to prepare the americium fraction for counting by alpha spectrometry. The method uses vacuum-assisted flow to improve the speed of separations. The sample is fused using procedures described in Section 6.3.3 for concrete and brick, Section 6.3.7 for asphalt matrix samples, Section 6.3.8 for asphalt roofing matrix samples and Section 6.3.9 for limestone samples. The americium isotopes are removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. Americium-243 tracer, added to the sample, is used as a yield monitor. The STS is prepared by microprecipitation with cerium (III) fluoride.

The method is capable of achieving a required method uncertainty for Am-241 of 0.20 pCi/g at an analytical action level of 1.5 pCi/g, using a sample weight of approximately 1 g and a count time of at least 4 hours.

Special Considerations: Alpha-emitting radionuclides with irresolvable alpha energies, such as plutonium-238 (5.50 MeV) and thorium-228 (5.42 MeV), can interfere with measurement of americium-241 and must be chemically separated to enable measurement. This method separates these radionuclides effectively. The significance of peak overlap is determined by the detector's alpha energy resolution characteristics and the quality of the final precipitate that is counted. A thorium removal rinse is performed on DGA resin in the event that any thorium ions pass through TEVA resin onto DGA resin. A dilute nitric acid rinse is performed to remove calcium and lanthanum ions that could end up on the final alpha source filter as fluoride solids.

Non-radiological interferences include anions that can complex americium, such as fluoride and phosphate, and lead to lower yields. Higher valence anions (e.g., phosphate) may lead to lower yields when using the evaporation option due to competition with active sites on the resin. Boric acid added in the load solution complexes fluoride ions, while aluminum complexes both fluoride as well as any residual phosphate that may be present. Clays that are present in some decontamination agents can contain iron, magnesium and calcium, which can be released as ions, via ion exchange, in the presence of certain radionuclides and cause interferences. High levels of calcium can have an adverse impact on americium retention on DGA resin. This interference is minimized by increasing the nitrate concentration to lower calcium retention and increase americium affinity. High levels of iron, manganese or magnesium can also have an impact on exchange site availability and/or poisoning of the Eichrom extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-007. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.7 Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt samples
Method Selected for: This method has been selected for preparation of americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt matrices.

Description of Method: The method is based on heating a representative, finely milled 1- to 1.5-g aliquant asphalt sample to remove organic components, followed by rapid fusion using sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation), followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a phosphate precipitation, followed by a calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. The method is applicable to the sodium hydroxide fusion of asphalt samples, prior to the chemical separation procedures described in the following methods:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: Asphalt samples with larger particle size may require a longer fusion time. Information regarding the elemental composition of the sample may be helpful to determine any native concentrations of uranium, radium, thorium, strontium or barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In those samples where native constituents are present that could interfere with the determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of these constituents in advance of chemical separation (using a separate aliquant of fused material) and to make appropriate adjustments to the yield calculations or amount of carrier added. Aluminum nitrate reagent typically contains trace levels

of uranium contamination. To achieve the lowest possible blanks for isotopic uranium measurements, the aluminum nitrate reagent can be passed through ~7 mL TRU resin or UTEVA resin. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R16-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.8 Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Material Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt roofing material samples
Method Selected for: This method has been selected for preparation of americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt shingles.

Description of Method: Asphalt roofing material samples should be cut into very small pieces prior to taking a representative aliquant for furnace heating and fusion. The method is based on ashing a 25-g subsample of asphalt roofing material sample in a furnace to remove organic components, followed by taking a representative aliquant from the ashed sample. A 1- to 1.5-g aliquant is fused using sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a phosphate precipitation, followed by a calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. The method is applicable to the sodium hydroxide fusion of asphalt shingle samples, prior to the chemical separation procedures described in the following methods:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental

Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: The term "asphalt roofing materials" is used in this procedure to mean asphalt organic shingles or asphalt fiberglass shingles typically used for residential or commercial roofs. This roofing material procedure was validated with asphalt fiberglass shingles. Roofing material samples should be cut into very small pieces prior to taking a representative aliquant for furnace heating and fusion.

Bitumen components, which may have affinity for the radionuclides, are destroyed in this method. Radionuclides deposited on the surface of the asphalt roofing material are effectively digested, including refractory radionuclide particles. A small amount of mineralized granules may remain after the fusion. Information regarding the elemental composition of the sample may be helpful. For example, asphalt roofing materials may have native concentrations of uranium, radium, thorium, stable strontium or stable barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In those samples where constituents are present that could interfere with the determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of these constituents in advance of chemical separation (using a separate aliquant of fused material) and make appropriate adjustments to the yield calculations or amount of carrier added. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Air and Radiation Environmental Laboratory. August 2016. "Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Material Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R16-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.9 Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90,

uranium-234, uranium-235 and uranium-238 in limestone samples.
Method Selected for: This method has been selected for preparation of americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in limestone samples.

Description of Method: Limestone samples may be received as core samples, crushed samples, or pieces of various sizes. The samples should be crushed and pulverized (milled) to achieve a particle size small enough that representative subsamples can be taken and representative aliquants analyzed. The method is based on the rapid dissolution of representative, finely milled aliquants of approximately 1 g of limestone using sodium hydroxide fusion at 600 °C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron hydroxide/titanium hydroxide precipitation followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a phosphate precipitation followed by a calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. The method is applicable to the sodium hydroxide fusion of limestone samples, prior to the chemical separation procedures described in the following:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: Limestone samples with larger particle sizes may require a longer fusion time. Samples with elevated activity or samples that require multiple analyses from a single aliquant may need to be split after dissolution. In these cases, the initial digestate and the split fractions should be measured carefully to ensure that the sample aliquant for analysis is accurately determined.

Limestone may have native concentrations of uranium, radium, thorium, strontium or barium, any of which may have an effect on the chemical separations used following the fusion of the sample. In some cases (e.g., strontium analysis), elemental analysis of the digestate prior to chemical separations may be necessary to determine native concentrations of carrier elements. The amount of stable strontium added to limestone samples is designed to minimize the impact from native stable strontium.

Additional information regarding potential interferences and procedures for addressing the interferences is provided in Section 4 of the method.

Source: U.S. EPA, National Air and Radiation Environmental Laboratory. August 2018. "Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R-18-002. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

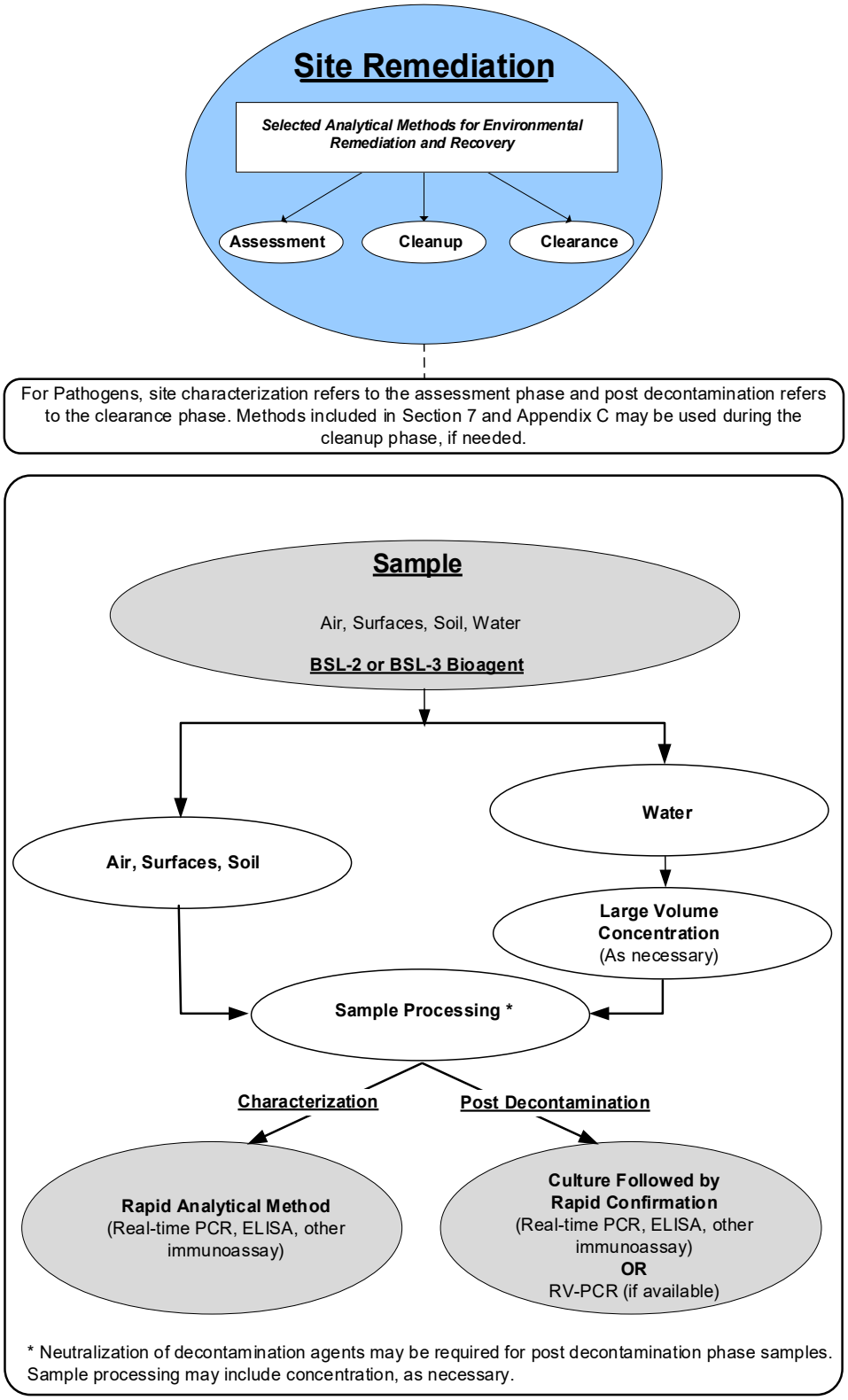
Section 7.0: Selected Pathogen Methods

Following a wide-area microbial contamination incident of national significance, it is assumed that the identification, confirmation and strain-level characterization of the pathogen have been completed before the U.S. Environmental Protection Agency's (EPA) remediation actions begin. The first phase of EPA's actions includes site characterization, to determine the extent and magnitude of contamination and to guide remediation planning. Based on the results of sample analyses for site characterization, EPA will determine the approach for site decontamination. During the post decontamination (clearance) phase of remediation, samples are collected and analyzed to determine the efficacy of the decontamination treatment.

The purpose of this section is to provide guidance to stakeholders in determining the appropriate methods for each remedial phase (site characterization and/or post decontamination) of a response to a contamination incident. Emphasis is given to the following environmental sample types: air, surfaces, soils and water.

Selection of methods from Appendix C should be based on specific data and information needs, including consideration of the remediation phase and whether there is a need to determine either the presence of a pathogen, the viability of a pathogen or both. The flow chart in Figure 7-1 presents a summary of the sample types, overall steps in sample analysis, and analytical techniques that should be used to address pathogens during EPA site remediation activities following a contamination incident. As depicted in Figure 7-1, for pathogens, site characterization refers to the assessment phase, decontamination refers to the cleanup phase, and post decontamination refers to the clearance phase. It is important to note that, in some cases, the procedures may not be fully developed or validated for each environmental sample type/pathogen combination listed in Appendix C.

Figure 7-1. Sample Analysis During Site Characterization and Post Decontamination Phases Following a Biological Contamination Event



Methods for Site Characterization Phase: Since decontamination of the affected site has to quickly follow the site characterization phase, rapid analytical methods should be selected to determine the extent and magnitude of contamination. It is assumed here that, prior to site characterization, the identity and viability of the pathogen have been determined. Therefore, in most cases, the analytical methods selected for the site characterization phase may not have to determine the viability of the pathogen. The methods chosen should also provide a high throughput analytical capability, so that a large number of samples can be rapidly analyzed to determine the presence or absence of the pathogen and allow for site decontamination planning in a time-efficient manner. For most pathogens, such methods routinely include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) or other immunoassay-based methods. Depending on the pathogen, type of incident and response, culture methods could be appropriate for use during site characterization. In certain cases, the determination of the extent of pathogen contamination within this phase may drive decontamination planning.

Methods for Post Decontamination Phase: It is extremely critical that the analytical methods used for sample analysis during the post decontamination phase be highly sensitive, specific, rapid and able to determine pathogen viability. For post decontamination phase samples, neutralization or removal of the decontamination agent may be required prior to analysis to minimize false negative results. Traditional microbiological culture methods typically include plating on selective medium to determine the viability of the pathogen and to minimize or eliminate non-target growth. The absence of growth on the medium generally indicates the absence of live pathogens in the sample (with the exception of some pathogens which may become viable but non-culturable [VBNC]). To minimize the analytical time needed to obtain results, typical colonies should be quickly analyzed to confirm the presence of the pathogen using reliable and rapid methods such as PCR, ELISA or other immunoassay-based methods, as opposed to time and labor intensive traditional biochemical and serological procedures. For *Bacillus anthracis* (Létant et al. 2011[12], U.S. EPA 2011[13], U.S. EPA 2017[14]), *Francisella tularensis* (U.S. EPA 2019[15]) and *Yersinia pestis* (U.S. EPA 2016[16]), the Rapid Viability-PCR (RV-PCR) method may be used because it provides rapid and high throughput sample analysis results in addition to viability determination.

A list of methods that have been selected by the Pathogen Methods Work Group for use in analyzing environmental samples for pathogens is provided in Appendix C. These methods should be used during remediation activities following a contamination incident. Appendix C is sorted alphabetically within pathogen categories (i.e., bacteria, viruses, protozoa and helminths). Protocols from peer-reviewed journal articles are listed where verified and/or validated methods for pathogens are not currently available. The literature references will be replaced as fully developed and validated protocols become available.

***Please note*:** This section provides guidance for selecting pathogen methods to facilitate data comparability when laboratories analyze a large number of samples during remediation. Not all methods have been verified for the pathogen/sample type combinations listed in Appendix C. Please refer to the specified method to identify analyte/sample type combinations for which the method has been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

[12] Létant, S. E., Murphy, G.A., Alfaro, T. M., Avila, J. R., Kane, S. R., Raber, E., Bunt, T. M. and Shah, S. R. 2011. "Rapid-Viability PCR Method for Detection of Live, Virulent *Bacillus anthracis* in Environmental Samples." *Applied Environmental Microbiology*. 77(18): 6570–6578.
[13] U.S. EPA. 2011. "Development and Verification of Rapid Viability Polymerase Chain Reaction (RV-PCR) Protocols for *Bacillus anthracis* – For Application to Air Filters, Water and Surface Samples." EPA/600/R-10/156.
[14] U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
[15] U.S. EPA. 2019. "Protocol for Detection of *Francisella tularensis* in Environmental Samples During the Remediation Phase of a Tularemia Incident" (EPA FT Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-19/110.
[16] U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109.

Pathogens that require biosafety level (BSL)-4 containment and practices, such as hemorrhagic fever viruses and Variola major (smallpox) will be handled only by reference laboratories with BSL-4 capability and are not included in this document. All other pathogens should be handled using BSL-2 or BSL-3 containment and practices, as appropriate. If known, the BSL classification for each pathogen is provided in the method summaries in Sections 7.2 through 7.5. Pathogens that are considered to be solely of agricultural concern (i.e., animal and plant pathogens) are not currently included. However, such pathogens may be considered for possible inclusion in future revisions.

Culture-based methods have been selected for many of the pathogens; however, due to technical difficulty and time constraints, molecular techniques such as PCR will likely be used for viruses.

Some of the methods in Appendix C include multiple analytical techniques by inference. The analytical technique listed in Appendix C for each pathogen is intended to be a description of the predominant technique that is required to provide the data quality parameter (viability or detection and identification). This description does not preclude the use of other techniques that are within or referenced by the method. For example, a viability method or procedure listed as "culture" might include immunochemical or PCR-based assays for the identification and/or confirmation of isolates. Several of the methods listed in Appendix C also include options such as the use of multiple cell culture media for primary isolation and a selection of a defined subset of biochemical tests for confirmation. To expedite time-to-results, however, isolates should be confirmed using rapid techniques (e.g., PCR, ELISA).

Appendix C includes the following information:

- **Pathogen(s).** A specific causative agent (e.g., viruses, bacteria) of disease.
- **Analytical technique.** An analytical procedure used to determine the identity, quantity and/or viability of a pathogen.
- **Method type.** Two method types (sample processing and analytical) are used to complete sample analysis. In some cases, a single method contains information for both sample processing and the analytical procedure. In most instances, however, two separate methods may need to be used.
- **Analytical method.** A series of techniques which together isolate, concentrate and detect a microorganism or group of microorganisms. In some cases, a unique identifier or number is assigned to an analytical method by the method publisher. Analytical methods can be developed for various sample types, including:
  - **Air (air filters, impingers, impactor media, collection fluid).** The recommended method/procedure for the pathogen of interest in air samples.
  - **Surfaces (swabs, wipes, Sponge-Sticks, filter cassettes).** The recommended method/procedure for the pathogen of interest on surfaces.
  - **Soil.** The recommended method/procedure for the pathogen of interest in soils.
  - **Water (surface water, drinking water, wastewater, post decontamination wastewater).** The recommended method/procedure for the pathogen of interest in water (concentrated and small volume grab samples). *Note*: additional sample processing may be required for wastewater samples to remove solids (see CDC's webpage for additional information on processing wastewater samples for viruses: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html).

Sample Processing: Sample processing can include recovery of the target contaminant from the sample, cleanup to remove potential interferents, and concentration of the target contaminant. It is widely recognized in the scientific community that the processing of biologically contaminated environmental samples is one of the most challenging aspects of sample analysis. Although details regarding sample processing are not included, it is critical that end users select the most appropriate sample processing procedure for a given sample type and analytical method. It is highly unlikely that a single procedure will

be applicable to all sample types and analytical methods. Inadequate sample processing may not only decrease recovery efficiency of biological targets (e.g., pathogen, deoxyribonucleic acid/ribonucleic acid [DNA/RNA], antigen/protein) from the samples, but also prevent accurate quantitation and high throughput. Samples should not be stored indefinitely and should be processed and analyzed as soon as possible upon receipt. ***Note***: For post decontamination samples it may be necessary to neutralize the decontamination agent.

The methods listed attempt to address multiple environmental sample types, each with different physical, chemical and biological properties (e.g., pH, inhibitory substances and background microorganisms). In this edition, emphasis is given to the environmental sample types that are predominately used to fulfill EPA's responsibilities following a contamination incident (e.g., air, surfaces, soils, water). Other sample types may have to be analyzed, and for those sample types, specific requests should be sent to the Pathogen Methods Lead and Alternate Lead (see Section 4.0).

7.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given pathogen as well as recommendations for quality control (QC) procedures.

Additional information on the pathogens listed in Appendix C can be found in the Centers for Disease Control and Prevention's (CDC's) Emergency Preparedness and Response website (https://emergency.cdc.gov/bioterrorism/index.asp) and the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) 2012, "Bad Bug Book" (https://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/).

In some cases, the availability of reagents and standards required for the selected analytical methods might be limited. In these cases, the pathogen methods points of contact listed in Section 4.0 should be contacted for additional information.

7.1.1 Standard Operating Procedures for Identifying Pathogen Methods

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected pathogen methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers pertain only to technical aspects of method usability, and do not pertain to aspects such as cost, equipment availability and sample throughput.

Tier I: The method was developed for the pathogen and sample type. The method has been evaluated by multiple laboratories, a detailed protocol has been developed, and suitable QC measures and checks are provided. (Examples: EPA Method 1623.1 [*Cryptosporidium* in water]; Standard Methods 9260 E [*Shigella* culture method].)

Tier II: The pathogen is the target of the method, and the method has been evaluated by one or more laboratories. The available data and/or information indicate that additional testing and/or modifications will likely be needed. (Example: Cunningham et al. 2010 [*Shigella* molecular method].)

Tier III: The pathogen is not the target of the method but the method is for the specific sample type and the pathogen is similar to the target of the method (i.e., vegetative bacteria, spore-forming bacteria, virus or protozoa). Data and expert opinion suggest, however, that the

method(s) may be applicable with modifications. (Example: EPA *Yersinia pestis* protocol for *Chlamydophila psittaci* in water.)

To determine the appropriate analytical method that is to be used for an environmental sample, locate the pathogen in Appendix C: Selected Pathogen Methods, under the "Pathogen(s)" column. After locating the pathogen, continue across the table and select an analytical technique. After an analytical technique has been chosen (e.g., culture, PCR, immunoassay), select the analytical method applicable to the sample type of interest (air, surfaces, soil, water).

Once a method has been identified in Appendix C, the corresponding method summary can be found in Sections 7.2 through 7.5. Method summaries are listed in alphabetical order within each pathogen subcategory (i.e., bacteria, virus, protozoa, helminths) and then by order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), and literature references. Where available, a direct link to the full text of the method is provided with the method summary. For additional information regarding sample processing and analysis procedures available through consensus standards organizations, other federal agencies, and journals, please use the source information provided in **Table 7-1**.

Table 7-1. Sources of Pathogen Methods

Name*	Publisher	Reference
African Journal of Medicine and Medical Sciences	College of Medicine, University of Ibadan	http://www.ojshostng.com/index.php/ajmms
Agriculture & Environmental Letters	Wiley	https://acsess.onlinelibrary.wiley.com/journal/24719625
American Journal of Tropical Medicine and Hygiene	American Society of Tropical Medicine and Hygiene	https://www.ajtmh.org/
American Journal of Veterinary Research	American Veterinary Medical Association	https://avmajournals.avma.org/view/journals/ajvr/ajvr-overview.xml
Antimicrobial Agents and Chemotherapy	American Society for Microbiology (ASM)	http://aac.asm.org/
Applied and Environmental Microbiology	ASM	http://aem.asm.org/
Applied Biosafety	Mary Ann Liebert, Inc.	https://home.liebertpub.com/publications/applied-biosafety/661/
Archives of Virology	Springer	http://link.springer.com/journal/705
Bacteriological Analytical Manual (BAM)	FDA CFSAN	http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
BMC Microbiology	Springer	http://www.springer.com/life+sciences/microbiology/journal/12866
Canadian Journal of Microbiology	Canadian Science Publishing	https://cdnsciencepub.com/loi/cjm
Clinical Chemistry	American Association for Clinical Chemistry	https://academic.oup.com/clinchem/issue
Clinical Infectious Diseases	Oxford	https://cid.oxfordjournals.org/
Current Protocols in Microbiology	Wiley	http://onlinelibrary.wiley.com/book/10.1002/9780471729259
Emerging Infectious Diseases	CDC	http://wwwnc.cdc.gov/eid/

Name*	Publisher	Reference
Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage and Sludge	EPA, National Risk Management Research Laboratory (NRMRL)	https://www.epa.gov/biosolids/control-pathogens-and-vector-attraction-sewage-sludge
Environmental Science and Technology	American Chemical Society (ACS)	http://pubs.acs.org/journal/esthag
EPA Analytical Protocols	EPA, CESER (formerly NHSRC)	https://www.epa.gov/esam/esam-collaborative-analytical-methods-and-protocols-pathogens
EPA Microbiology Home Page	EPA	https://www.epa.gov/cwa-methods
International Organization for Standardization (ISO) Methods	ISO	http://www.iso.org/iso/home.html
Journal of Clinical Microbiology	ASM	http://jcm.asm.org/
Journal of Food Protection	International Association for Food Protection	https://www.foodprotection.org/publications/journal-of-food-protection/
Journal of Medical Virology	Wiley	http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1096-9071
Journal of Microbiological Methods	Elsevier	http://www.sciencedirect.com/science/journal/01677012
Journal of Parasitology	American Society of Parasitologists	http://www.journalofparasitology.org/
Journal of Parasitology Research	Hindawi Publishing Corporation	https://www.hindawi.com/journals/jpr/contents/
Journal of Virological Methods	Elsevier	http://www.sciencedirect.com/science/journal/01660934
Legionella: Methods and Protocols, Methods in Molecular Biology	Springer	http://link.springer.com/book/10.1007%2F978-1-62703-161-5
Methods in Molecular Biology	Springer	http://link.springer.com/search?facet-series=7651&facet-content-type=Book
Molecular and Cellular Probes	Elsevier	http://www.sciencedirect.com/science/journal/08908508
Neglected Tropical Diseases	PLoS	http://journals.plos.org/plosntds/
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov
Parasitology	Cambridge University Press	https://www.cambridge.org/core/journals/parasitology
Parasitology Research	Springer	http://www.springer.com/biomed/medical+microbiology/journal/436
Pathogens and Disease	Wiley	https://academic.oup.com/femspd
PLoS ONE	PLoS	https://journals.plos.org/plosone/
Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases	ASM	https://asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C
Science of the Total Environment	Elsevier	https://www.journals.elsevier.com/science-of-the-total-environment
Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2017	American Public Health Association (APHA)	http://www.standardmethods.org
Transactions of the Royal Society of Tropical Medicine and Hygiene	Oxford	http://trstmh.oxfordjournals.org/

Name*	Publisher	Reference
U.S. Department of Agriculture (USDA) *Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook*	USDA FSIS	https://www.fsis.usda.gov/news-events/publications/microbiology-laboratory-guidebook
U.S. Department of Health and Human Services *Procedures for the Recovery of Legionella from the Environment*	CDC	https://www.cdc.gov/legionella/labs/procedures-manual.html

* Subscription and/or purchase may be required. ***Note***: ASM does not require a subscription or purchase 6 months after the publication date.

7.1.2 General QC Guidelines for Pathogen Methods

Generation of analytical data of known and documented quality is a critical factor in the accurate assessment of and appropriate response to emergency situations. The generation of data of sufficient quality requires that analytical laboratories: (1) have appropriately trained and proficient personnel; (2) acquire and maintain required supplies, equipment and reagents; (3) conduct the appropriate QC procedures to ensure that all measurement systems are in control and operating properly; (4) properly document all analytical results; (5) properly document analytical QC procedures and corrective actions; (6) conduct training and proficiency testing; and (7) maintain personnel training and proficiency testing records.[17]

The level or amount of QC needed depends on the intended purpose of the data generated. Specific data needs should be identified and QC requirements, based on those needs, applied consistently across laboratories when multiple laboratories are used. The individual methods listed, sampling and analytical protocols or contractual statements of work should be consulted to determine if additional QC procedures are required.

Method-specific QC requirements are described in many of the methods cited in this manual and will be included in protocols developed to address specific pathogen/sample type combinations of concern. In general, analytical QC requirements for pathogen methods include an initial demonstration of measurement system capability, as well as the capability of the laboratory and the analyst to perform the method with the required precision and accuracy. In addition, for molecular techniques (e.g., PCR) general guidelines are provided in EPA's 2004 "Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples" (Cincinnati, OH: U.S. EPA. EPA 815-B-04-001) at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf.

Ongoing analysis of control samples to ensure the continued reliability of the analytical results should also be performed. At a minimum, the following QC analyses should be conducted on an ongoing basis:

- Media and reagent sterility checks
- Positive and negative controls
- Method blanks
- Reference matrix spikes to evaluate initial and ongoing method/analyst performance, if available
- Matrix spikes (where possible) to evaluate method performance in the sample type of interest
- Matrix spike duplicates (MSD) and/or sample replicates to assess method precision
- Sample processing controls to evaluate processing procedures (e.g., extraction, concentration) in the sample type of interest
- Instrument calibration checks and temperature controls

[17] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality/guidance-systematic-planning-using-data-quality-objectives-process-epa-qag-4.

QC procedures and proper calibration and maintenance of ancillary laboratory equipment (e.g., thermometers, autoclaves, pipettors) should be performed as frequently as necessary to ensure the reliability of analytical results.

***Please note*:** The type and quantity of appropriate quality assurance (QA) and QC procedures that will be required are incident-specific and should be included in incident-specific documents (e.g., Quality Assurance Project Plan [QAPP], Sampling and Analysis Plan [SAP], laboratory Statement of Work [SOW], analytical methods). This documentation and/or Incident Command should be consulted regarding appropriate QA and QC procedures prior to sample analysis.

7.1.3 Safety and Waste Management

Laboratories should have a documented health and safety plan for handling samples that might contain target chemical, biological and/or radiological (CBR) contaminants. Laboratory staff should be trained in the safety and waste handling procedures included in the plan and implement those procedures. Pathogens in samples taken from areas contaminated as the result of a homeland security event may be more hazardous than naturally occurring pathogens of the same genus and species. The pathogens may have been manufactured, engineered or treated to enhance dispersion or virulence characteristics. Laboratories should carefully consider implementing additional safety measures before agreeing to accept these samples. Sample disposal should follow federal and local regulations.

In addition, many of the methods listed in Appendix C and summarized or cited in Sections 7.2 through 7.5 contain specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. BSL-3 is applicable when performing manipulations of indigenous or exotic agents that can cause serious or potentially lethal disease and also have the potential for aerosol transmission. Whenever available, BSLs are provided in the method summaries in Sections 7.2 through 7.5. However, some pathogens that are normally handled at BSL-2 may require BSL-3 procedures and facilities if large volumes, high concentrations or potential aerosols are expected as a part of the analytical process. For more information on BSL practices and procedures, the following references should be consulted:

- CDC. 2020. "Biosafety in Microbiological and Biomedical Laboratories" (BMBL), 6th Edition. Available at: https://www.cdc.gov/labs/BMBL.html
- CDC. 2002. "Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents." *Morbidity and Mortality Weekly Report*, Vol. 51, No. RR-19, 1-6, December 6, 2002. Available at: http://www.cdc.gov/mmwr/pdf/rr/rr5119.pdf
- Select Agent Rules and Regulations found at the National Select Agent Registry. Available at: http://www.selectagents.gov/ and https://www.selectagents.gov/regulations/index.htm

The following sources provide information regarding waste management:

- U.S. EPA – Hazardous Waste Management (40 CFR part 260) and U.S. EPA Administered Permit Programs (40 CFR part 270). Available at: http://www.ecfr.gov/
- U.S. EPA. 2010. Laboratory Environmental Sample Disposal Information Document Companion to Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM) Revision 5. EPA/600/R-10/092. Available at: http://www.epa.gov/sites/production/files/2015-06/documents/lesdid.pdf

Other resources that can be consulted for additional information include the following:

- OSHA – Hazardous Waste Operations and Emergency Response (29 CFR part 1910.120). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9765
- OSHA – Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR part 1910.1450). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106
- OSHA – Respiratory Protection (29 CFR part 1910.134). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=12716&p_table=STANDARDS
- DOT Hazardous Materials Shipment and Packaging (49 CFR parts 171–180). Available at: http://www.ecfr.gov/cgi-bin/text-idx?gp=&SID=994b04d45ee6d584ce676138929280b3&mc=true&tpl=/ecfrbrowse/Title49/49tab_02.tpl

7.1.4 Laboratory Response Network (LRN)

The LRN is a national network of local, state, federal, military, food, agricultural, veterinary and environmental laboratories that was created in accordance with Presidential Decision Directive 39, which established terrorism preparedness responsibilities for federal agencies. The CDC provides technical and scientific support to member laboratories as well as secure access to standardized procedures (e.g., sample processing, culture, immunoassay, PCR) and reagents for rapid (4–6 hours) presumptive detection of select agents. The algorithm for a confirmed result is often a combination of one or more presumptive positive results from a rapid assay in combination with a positive result from one of the "gold standard" methods, such as culture. The standardized procedures, reagents and agent-specific algorithms are considered to be sensitive and are available only to LRN member laboratories. Thus, these procedures are not available to the general public and are not discussed in this document. However, EPA has published methods for the analysis of *Bacillus anthracis, Yersinia pestis* and *Francisella tularensis* in environmental matrices that are included in this document.

It is important to note that, in some cases, the procedures may not be fully developed or validated for each environmental sample type/pathogen combination listed in Appendix C. Except for *Coxiella burnetii*, culture methods are available for all of these pathogens as American Society for Microbiology's (ASM) Sentinel Laboratory Guidelines (available at: https://asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C).

The pathogens identified below and listed in Appendix C are included in the U.S. Health and Human Services (HHS)/U.S. Department of Agriculture (USDA) select agent list and should be analyzed in accordance with appropriate regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121, available at http://www.ecfr.gov/cgi-bin/ECFR?page=browse) and safety and BSL requirements (see CDC's BMBL, 6th Edition, available at: https://www.cdc.gov/labs/BMBL.html.

Select Agents Listed in Appendix C

Pathogen [Disease]	Agent Category
Bacillus anthracis [Anthrax]	Bacteria
Brucella spp. [Brucellosis]	Bacteria
Burkholderia mallei [Glanders]	Bacteria
Burkholderia pseudomallei [Melioidosis]	Bacteria
Coxiella burnetii [Q-fever]	Bacteria
Francisella tularensis [Tularemia]	Bacteria
Yersinia pestis [Plague]	Bacteria

For additional information on the LRN, including selection of a laboratory capable of receiving and processing the specified sample type/pathogen, please use the contact information provided below or visit https://emergency.cdc.gov/lrn/.

Centers for Disease Control and Prevention (CDC)
Laboratory Preparedness and Response Branch
Division of Preparedness and Emerging Infection
National Center for Emerging, Zoonotic and Infectious Disease
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
E-mail: lrn@cdc.gov
Website: https://emergency.cdc.gov/lrn/contact.asp

Local public health laboratories, private laboratories and commercial laboratories with questions about the LRN should contact their state public health laboratory director or the Association of Public Health Laboratories (APHL) (contact information provided below).

Association of Public Health Laboratories
8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Telephone: (240) 485-2745
Fax: (240) 485-2700
Website: http://www.aphl.org
E-mail: info@aphl.org

The following references and information sources provide additional information regarding Select Agents Culture Methods – LRN Sentinel Labs (website references for individual pathogens are included in their respective summaries):

- Avian Influenzae: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/Novel-Influenza.pdf
- *Brucella*: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/Brucella-2016-March.pdf
- *Burkholderia mallei* and *B. pseudomallei*: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/Burkholderia-Marc2016.pdf
- *Coxiella burnetii*: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/Coxiella316-photos.pdf

- *Francisella tularensis*: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/tularemia.pdf
- *Yersinia pestis*: https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Y-pestis-fixed-figures.pdf

Sources:

ASM. 2013. Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases. Available via: https://asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C

CDC. 2020. "Biosafety in Microbiological and Biomedical Laboratories" (BMBL), 6th Edition. https://www.cdc.gov/labs/BMBL.html

7.2 Method Summaries for Bacteria

Summaries for the analytical methods listed in Appendix C are provided in Sections 7.2.1 through 7.2.17. Each summary contains a brief description of the analytical methods selected for each bacterial pathogen, and links to, or sources for, obtaining full versions of the methods. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

7.2.1 *Bacillus anthracis* [Anthrax] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.1.1
Post Decontamination	Rapid Viability-PCR (RV-PCR)	7.2.1.2
	Culture and Real-Time PCR	7.2.1.3

[1] See Appendix C for corresponding method usability tiers.

7.2.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water.

Sample Processing:

- Soil samples should be processed according to Silvestri et al. 2016 (Tier II).
- All other environmental sample types should be processed according to EPA's "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (U.S. EPA 2017, Tier I), referred to as the "EPA BA Protocol".

Analytical Technique: Real-time PCR (U.S. EPA 2017, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2 [U.S. EPA 2017]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *B. anthracis* spores. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." *Journal of Microbiological Methods*. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

7.2.1.2 Post Decontamination Sample Analyses (RV-PCR)

Note: Laboratories without RV-PCR capability should analyze samples according to the culture procedure provided in Section 7.2.1.3.

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water.

Sample Processing:

- Soil samples should be processed according to Silvestri et al. 2016 (Tier II).
- All other environmental sample types should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier I).

Analytical Technique: RV-PCR (U.S. EPA 2017, Tier I)

Description of Method: The RV-PCR procedure is a combination of a broth culture and real-time PCR. Culturing the sample allows the germination of *Bacillus anthracis* spores recovered from a processed sample. The real-time PCR provides rapid detection of *Bacillus anthracis.* By combining both culture and PCR, the protocol allows for the detection of viable *Bacillus anthracis* spores. Prior to analysis, samples (e.g., air, surfaces, soils, water) are processed using multiple extraction and wash steps. After brain heart infusion broth is added to the spores, an aliquot (Time 0 [T_0]) is removed and stored at 4°C. The remaining broth is then incubated for 9 to 15 hours at 37°C. After the incubation, an aliquot is removed (Time Final [T_f]). Both T_0 and T_f aliquots then go through DNA extraction and purification followed by real-time PCR analysis. The cycle threshold (C_T) values for the T_0 and T_f aliquots are then compared. The difference in C_T values between the T_0 and T_f is used to detect viable *Bacillus anthracis* spores. A change (decrease) in the PCR $C_T \geq 6$ represents 2-log increased DNA concentration in the T_f aliquot relative to the T_0 aliquot, which in turn, represents an increase in DNA as a result of the germination and growth of viable spores in the sample during the incubation period. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." *Journal of Microbiological Methods*. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

7.2.1.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water.

Sample Processing:

- Soil samples should be processed according to Silvestri et al. 2016 (Tier II).
- All other environmental sample types should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier I).

Analytical Technique: Culture and real-time PCR (U.S. EPA 2017, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the sample is streaked for isolation onto tryptic soy agar with 5% sheep's blood. Plates are incubated at 35°C to 37°C for 18–24 hours. Isolated typical colonies are resuspended in sterile distilled water. The bacterial suspensions are then heated at 95°C to 98°C to release the DNA from the cells (EPA BA Protocol, Section 11 [U.S. EPA 2017]). DNA extracts are then used in real-time PCR to confirm the presence of *Bacillus anthracis*. Combining the culture component with confirmation using real-time PCR analyses allows for detection and viability results within 24–30 hours as compared to traditional culture procedures that require a minimum of 48 hours. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." *Journal of Microbiological Methods*. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

7.2.2 *Brucella* spp. [Brucellosis] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.2.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Culture and Real-Time PCR	7.2.2.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to EPA's "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (U.S. EPA 2016, Tier III), referred to as the EPA YP Protocol.

Analytical Technique: Real-time PCR (Hinić et al. 2008, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Hinić et al. 2008 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Hinić et al. 2008). The use of real-time

PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Brucella* spp. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-time PCR Systems." *Journal of Microbiological Methods*. 75(2): 375-378. http://www.sciencedirect.com/science/article/pii/S0167701208002522

7.2.2.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: Culture (ASM 2016, Tier I) and real-time PCR (Hinić et al. 2008, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are plated directly on selective and non-selective agars and incubated at 35°C (5–10% carbon dioxide) for up to 7 days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Hinić et al. 2008 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR

primers, probes and assay parameters (Hinić et al. 2008). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Brucella* spp. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). EPA/600/R-16/109. Cincinnati, OH: U.S. EPA. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-Time PCR Systems." *Journal of Microbiological Methods*. 75(2): 375-378. http://www.sciencedirect.com/science/article/pii/S0167701208002522

ASM. 2016. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species." https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel Files/Brucella-2016-March.pdf

7.2.3 *Burkholderia mallei* [Glanders] – BSL-3 and *Burkholderia pseudomallei* [Melioidosis] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.3.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Culture and Real-Time PCR	7.2.3.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.3.1 Site Characterization Sample Analysis (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to Hall et al. 2019 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: Real-time PCR (Tomaso et al. 2006 and Novak et al. 2006. Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (analytical technique references cited above or the EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Tomaso et al. 2006 and Novak et al. 2006). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Burkholderia mallei* and *Burkholderia pseudomallei*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:
Hall, C.M., Jaramillo, S., Jimenez, R., Stone, N.E., Centner, H., Busch, J.D., Bratsch, N., Roe, C.C., Gee, J.E., Hoffmaster, A.R., Rivera-Garcia, S., Soltero, F, Ryff, K., Perez-Padilla, J., Keim, P., Sahl, J.W., and Wagner, D.M. 2019. "*Burkholderia pseudomallei*, the causative agent of melioidosis, is rare but ecologically established and widely dispersed in the environment in Puerto Rico." *PLoS Neglected Tropical Diseases*. 13(9):e0007727. https://doi.org/10.1371/journal.pntd.0007727

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." *Clinical Chemistry*. 52(2): 307-310. https://doi.org/10.1373/clinchem.2005.059196

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." *Journal of Clinical Microbiology*. 44(1): 85-90. http://jcm.asm.org/content/44/1/85.full.pdf+html

7.2.3.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to Hall et al. 2019 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III)

Analytical Technique: Culture (ASM 2016, Tier I) and real-time PCR (Tomaso et al. 2006 and Novak et al. 2006, Tier II).

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are plated directly on sheep blood agar and incubated at 35°C–37°C for 48 hours. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (real-time PCR analytical techniques cited above or the EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Tomaso et al. 2006 and Novak et al. 2006). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Burkholderia mallei* and *Burkholderia pseudomallei*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:
Hall, C.M., Jaramillo, S., Jimenez, R., Stone, N.E., Centner, H., Busch, J.D., Bratsch, N., Roe, C.C., Gee, J.E., Hoffmaster, A.R., Rivera-Garcia, S., Soltero, F, Ryff, K., Perez-Padilla, J., Keim,

P., Sahl, J.W., and Wagner, D.M. 2019. "*Burkholderia pseudomallei*, the causative agent of melioidosis, is rare but ecologically established and widely dispersed in the environment in Puerto Rico." *PLoS Neglected Tropical Diseases*. 13(9):e0007727. https://doi.org/10.1371/journal.pntd.0007727

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

ASM. 2016. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases, Glanders: *Burkholderia mallei* and Melioidosis: *Burkholderia pseudomallei*." https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/Burkholderia-Marc2016.pdf

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." *Clinical Chemistry*. 52(2): 307-310. https://doi.org/10.1373/clinchem.2005.059196

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." *Journal of Clinical Microbiology*. 44(1): 85-90. http://jcm.asm.org/content/44/1/85.full.pdf+html

7.2.4 *Campylobacter jejuni* [Campylobacteriosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.4.1
Post Decontamination	Culture and Real-Time PCR	7.2.4.2

[1] See Appendix C for corresponding method usability tiers.

7.2.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil and water samples should be processed according to Hiett 2017 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: Real-time PCR (Cunningham et al. 2010, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Cunningham et al. 2010 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham et al. 2010). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Campylobacter jejuni*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Hiett, K.L. 2017. "*Campylobacter jejuni* Isolation/Enumeration from Environmental Samples" In: Butcher, J., Stintzi, A. *Campylobacter jejuni*. *Methods in Molecular Biology*. 1512:1-8. https://doi.org/10.1007/978-1-4939-6536-6_1

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." *Journal of Clinical Microbiology*. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.4.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil and water samples should be processed according to Hiett 2017 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: Culture (ISO 2019, Tier I) and real-time PCR (Cunningham et al. 2010, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are inoculated into broth media and incubated, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Cunningham et al. 2010 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham et al. 2010). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Campylobacter jejuni*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control*

Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Hiett, K.L. 2017. "*Campylobacter jejuni* Isolation/Enumeration from Environmental Samples" In: Butcher, J., Stintzi, A. *Campylobacter jejuni*. *Methods in Molecular Biology*. 1512:1-8. https://doi.org/10.1007/978-1-4939-6536-6_1

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." *Journal of Clinical Microbiology*. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

ISO. 2019. ISO 17995:2019 Water quality – Detection and Enumeration of Thermotolerant *Campylobacter* spp. https://www.iso.org/standard/69047.html

7.2.5 *Chlamydophila psittaci* [Psittacosis] (formerly known as *Chlamydia psittaci*) – BSL-2; BSL-3 for Aerosol Release

Remediation Phase	Analytical Technique [1]	Section
Site Characterization	PCR	7.2.5.1
Post Decontamination	Tissue Culture and PCR	7.2.5.2

[1] See Appendix C for corresponding method usability tiers.

7.2.5.1 Site Characterization Sample Analyses (PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: PCR (Madico et al. 2000, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Madico et al. 2000 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Madico et al. 2000). The use of PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of

Chlamydophila psittaci. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." *Journal of Clinical Microbiology*. 38(3): 1085-1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.5.2 Post Decontamination Sample Analyses (Tissue Culture and PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Analytical Technique: Tissue culture and PCR (Madico et al. 2000, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto buffalo green monkey kidney (BGMK) cells to increase sensitivity. Target nucleic acid should be extracted, purified (Madico et al. 2000 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Madico et al. 2000). The use of PCR analyses directly on isolates allows for rapid confirmation of *Chlamydophila psittaci*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing

analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." *Journal of Clinical Microbiology*. 38(3): 1085-1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.6 *Coxiella burnetii* [Q-fever] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.6.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Tissue Culture and Real-Time PCR	7.2.6.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.6.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or the EPA BA Protocol (Tier III, U.S. EPA 2017).
- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (ultrafiltration [UF], U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III)

Analytical Technique: Real-time PCR (Panning et al. 2008, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Panning et al. 2008 or EPA BA Protocol, Section 9.2 [U.S. EPA 2017]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Panning et al. 2008). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Coxiella burnetii*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-

Time PCR With Internal Control System and Automated DNA Preparation." *BMC Microbiology*. 8:77. http://www.biomedcentral.com/1471-2180/8/77

7.2.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Tissue culture (Raoult et al. 1991, Tier II) and real-time PCR (Panning et al. 2008, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto human erythroleukemia cells and incubated for 6 days at 37°C. Target nucleic acid should be extracted, purified (Panning et al. 2008 or EPA BA Protocol, Section 11.6 [U.S. EPA 2017]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Panning et al. 2008). The use of real-time PCR analyses directly on isolates allows for rapid confirmation of *Coxiella burnetii*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR With Internal Control System and Automated DNA Preparation." *BMC Microbiology*. 8:77. http://www.biomedcentral.com/1471-2180/8/77

Raoult, D. Torres, H. and Drancourt, M. 1991. "Shell-Vial Assay: Evaluation of a New Technique for Determining Antibiotic Susceptibility, Tested in 13 Isolates of *Coxiella burnetii*." *Antimicrobial Agents and Chemotherapy*. 35(10): 2070-2077. http://aac.asm.org/content/35/10/2070.long

7.2.7 *Escherichia coli* O157:H7 – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.7.1
Post Decontamination	Culture and Real-Time PCR	7.2.7.2

[1] See Appendix C for corresponding method usability tiers.

7.2.7.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1680 (U.S. EPA 2014, Tier I).
- Water samples should be processed according to the EPA *Escherichia coli* O157:H7 Protocol (referred to as the EPA EC Protocol [U.S. EPA 2010, Tier I]).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Sen et al. 2011, Tier II)
Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above) and enrichment, the target nucleic acid should be extracted, purified (Sen et al. 2011 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Sen et al. 2011). ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2014. "Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC." Washington, DC: U.S. EPA. EPA-821-R-14-009. https://www.epa.gov/sites/default/files/2019-08/documents/method_1680_2014.pdf

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water" (EPA EC Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-10/056. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." *Environmental Science and Technology*. 45(6): 2250-2256. http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.7.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1680 (U.S. EPA 2014, Tier I).
- Water samples should be processed according to the EPA EC Protocol (U.S. EPA 2010, Tier I).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (EPA EC Protocol [U.S. EPA 2010, Tier I]) and real-time PCR (Sen et al. 2011, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are cultured using multiple media and immunomagnetic separation (IMS) (EPA EC Protocol [U.S. EPA 2010]). Typical isolates are then confirmed using biochemical and serological tests. To expedite time to results, isolates should be confirmed using real-time PCR analyses. Target nucleic acid should be extracted, purified (Sen et al. 2011 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Sen et al. 2011). The use of real-time PCR analyses allows for rapid confirmation of *E. coli* O157:H7. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2014. "Method 1680: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC." Washington, DC: U.S. EPA. EPA-821-R-14-009. https://www.epa.gov/sites/default/files/2019-08/documents/method_1680_2014.pdf

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water" (EPA EC Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-10/056. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." *Environmental Science and Technology*. 45(7): 2250-2256. http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.8 *Francisella tularensis* [Tularemia] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.8.1
Post Decontamination	Rapid Viability-PCR (RV-PCR)[2]	7.2.8.2
	Culture and Real-Time PCR	7.2.8.3

[1] See Appendix C for corresponding method usability tiers.

[2] Only applicable for water samples.

7.2.8.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to EPA's "Protocol for Detection of *Francisella tularensis* in Environmental Samples During the Remediation Phase of a Tularemia Incident" (U.S. EPA 2019, Tier I), referred to as the "EPA FT Protocol."

Note: The EPA FT Protocol does not include ultrafiltration of large volume water samples. For ultrafiltration of large volume water samples, refer to the EPA YP Protocol (U.S. EPA 2016).

Analytical Technique: Real-time PCR (EPA FT Protocol 2019, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (EPA FT Protocol, Section 9.6 [U.S. EPA 2019]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Francisella tularensis*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also

be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2019. "Protocol for Detection of *Francisella tularensis* in Environmental Samples During the Remediation Phase of a Tularemia Incident" (EPA FT Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-19/110. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=348592

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.8.2 Post Decontamination Sample Analyses (RV-PCR)

Note: Laboratories without RV-PCR capability should analyze water samples according to the culture procedure provided in Section 7.2.8.3.

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of water samples.

Sample Processing: Water samples should be processed according to the EPA FT Protocol (U.S. EPA 2019, Tier I). ***Note***: The EPA FT Protocol does not include ultrafiltration of large volume water samples. For ultrafiltration of large volume water samples, refer to the EPA YP Protocol (U.S. EPA 2016).

Analytical Technique: RV-PCR (U.S. EPA 2019, Tier I)

Description of Method: The RV-PCR procedure is a combination of a broth culture and real-time PCR. Culturing the sample allows the growth of *Francisella tularensis* recovered from a processed sample. The real-time PCR provides rapid detection of *Francisella tularensis.* By combining both culture and PCR, the protocol allows for the detection of viable *Francisella tularensis*. Prior to analysis, samples are processed using multiple extraction and wash steps. After brain heart infusion broth with supplements is added to the cells, an aliquot (Time 0 [T_0]) is removed and stored at 4°C. The remaining broth is then incubated for 30 hours at 37°C. After the incubation, an aliquot is removed (Time Final [T_f]). Both T_0 and T_f aliquots then go through DNA extraction and purification followed by real-time PCR analysis. The cycle threshold (C_T) values for the T_0 and T_f aliquots are then compared. The difference in C_T values between the T_0 and T_f is used to detect viable *Francisella tularensis*. A change (decrease) in the PCR $C_T \geq 6$ represents a 2-log increased DNA concentration in the T_f aliquot relative to the T_0 aliquot, which in turn represents an increase in DNA as a result of the growth of viable cells in the sample during the

incubation period. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

U.S. EPA. 2019. "Protocol for Detection of *Francisella tularensis* in Environmental Samples During the Remediation Phase of a Tularemia Incident" (EPA FT Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-19/110. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=348592

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.8.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA FT Protocol (U.S. EPA 2019, Tier I).

Note: The EPA FT Protocol does not include ultrafiltration of large volume water samples. For ultrafiltration of large volume water samples, refer to the EPA YP Protocol (U.S. EPA 2016).

Analytical Technique: Culture and real-time PCR (U.S. EPA 2019, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are plated directly onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA FT Protocol, Section 10.5 [U.S. EPA 2019]), and analyzed using the referenced target-specific PCR

primers, probes and assay parameters. The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Francisella tularensis*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2019. "Protocol for Detection of *Francisella tularensis* in Environmental Samples During the Remediation Phase of a Tularemia Incident" (EPA FT Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-19/110. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=348592

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.9 *Legionella pneumophila* [Legionellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.9.1
Post Decontamination	Culture and Real-Time PCR	7.2.9.2

[1] See Appendix C for corresponding method usability tiers.

7.2.9.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to U.S. DHHS 2005 (Tier I).
- All other environmental sample types should be processed according to Kozak et. al., 2013 (Tier I).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (ISO Method ISO/TS 12869:2019, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (ISO Method ISO/TS 12869:2019 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Legionella pneumophila*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. Department of Health and Human Services. 2005. "Procedures for the Recovery of *Legionella* from the Environment." Atlanta, GA: CDC. https://www.cdc.gov/legionella/labs/procedures-manual.html

Kozak, N.A., Lucas, C.E. and Winchell, J.M. 2013. "Identification of *Legionella* in the Environment." *Legionella*: *Methods and Protocols, Methods in Molecular Biology*. 954: 3-25. https://www.ncbi.nlm.nih.gov/pubmed/23150387

ISO. 2019. ISO/TS 12869:2019 Water quality — Detection and quantification of *Legionella spp*. and/or *Legionella pneumophila* by concentration and genic amplification by quantitative polymerase chain reaction (qPCR). https://www.iso.org/standard/70756.html

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.9.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to U.S. DHHS 2005 (Tier I).
- All other environmental sample types should be processed according to Kozak et al. 2013 (Tier I).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Kozak et al. 2013, Tier I) and real-time PCR (ISO Method ISO/TS 12869:2019, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are cultured using multiple media (buffered charcoal yeast extract [BCYE] with polymyxin B, cycloheimide and vancomycin [BCYE PCV]; or BCYE with glycine, polymyxin B, cycloheximide and vancomycin [BCYE GPCV]). Typical isolates are then confirmed using serological tests. To expedite time to results, isolates should be confirmed using real-time PCR analyses. Target nucleic acid should be extracted, purified (ISO Method ISO/TS 12869:2019 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]) and analyzed using the referenced target-specific PCR primers, probes and assay parameters (ISO Method ISO/TS 12869:2019). The use of real-time PCR analyses allows for rapid confirmation of *Legionella pneumophila*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. Department of Health and Human Services. 2005. "Procedures for the Recovery of Legionella from the Environment." Atlanta, GA: CDC. https://www.cdc.gov/legionella/labs/procedures-manual.html

Kozak, N.A., Lucas, C.E. and Winchell, J.M. 2013. "Identification of *Legionella* in the Environment." *Legionella*: *Methods and Protocols, Methods in Molecular Biology*. 954: 3-25. https://www.ncbi.nlm.nih.gov/pubmed/23150387

ISO. 2019. ISO/TS 12869:2019 Water quality — Detection and quantification of *Legionella spp*. and/or *Legionella pneumophila* by concentration and genic amplification by quantitative polymerase chain reaction (qPCR). https://www.iso.org/standard/70756.html

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.10 *Leptospira interrogans* [Leptospirosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.10.1
Post Decontamination	Culture and Real-Time PCR	7.2.10.2

[1] See Appendix C for corresponding method usability tiers.

7.2.10.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Standard Method 9260 I (APHA et al. 2017, Tier I)
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Palaniappan et al. 2005, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Palaniappan et al. 2005 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Palaniappan et al. 2005). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Leptospira interrogans*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

APHA, AWWA and WEF. 2017. "Method 9260 I: *Leptospira*." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." *Molecular and Cellular Probes*. 19(2): 111-117. http://www.sciencedirect.com/science/article/pii/S0890850804000970

7.2.10.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Standard Method 9260 I (APHA et al. 2017, Tier I)
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Standard Method 9260 I [APHA et al. 2017, Tier I]) and real-time PCR (Palaniappan et al. 2005, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are inoculated into selective broth media and incubated for up to six weeks at 30°C. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Palaniappan et al. 2005 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Palaniappan et al. 2005). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Leptospira interrogans*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. https://www.epa.gov/sites/default/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." *Molecular and Cellular Probes*. 19(2): 111-117. http://www.sciencedirect.com/science/article/pii/S0890850804000970

APHA, AWWA and WEF. 2017. "Method 9260 I: *Leptospira*." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.11 *Listeria monocytogenes* [Listeriosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR[1]	7.2.11.1
Post Decontamination	Culture and Real-Time PCR	7.2.11.2

[1] See Appendix C for corresponding method usability tiers.

7.2.11.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil and water samples should be processed according to Iwu and Okoh 2020 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (USDA FSIS 2021, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above) and enrichment, the target nucleic acid should be extracted, purified (USDA FSIS 2021 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (USDA FSIS 2021). ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Iwu C.D. and Okoh, A.I. 2020. "Characterization of antibiogram fingerprints in *Listeria monocytogenes* recovered from irrigation water and agricultural soil samples." *PLoS ONE*. 15(2): e0228956. https://doi.org/10.1371/journal.pone.0228956

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

USDA, FSIS. 2021. "FSIS Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples." Chapter MLG 8.13 in *Microbiology Laboratory Guidebook*. Athens, GA: USDA. https://www.fsis.usda.gov/sites/default/files/media_file/2021-09/MLG-8.13.pdf

7.2.11.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil and water samples should be processed according to Iwu and Okoh 2020 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Hitchins et al. 2017, Tier I) and real-time PCR (USDA FSIS 2021, Tier I)

Description of Method: Following appropriate sample processing (see Sample Processing procedures above), samples are inoculated into broth media, incubated for 48 hours, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (USDA FSIS 2021 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (USDA FSIS 2021). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Listeria monocytogenes*.
Note: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Iwu C.D. and Okoh, A.I. 2020. "Characterization of antibiogram fingerprints in *Listeria monocytogenes* recovered from irrigation water and agricultural soil samples." *PLoS ONE*. 15(2): e0228956. https://doi.org/10.1371/journal.pone.0228956

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

USDA, FSIS. 2021. "FSIS Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples." Chapter MLG 8.12 in *Microbiology Laboratory Guidebook*. Athens, GA: USDA. https://www.fsis.usda.gov/sites/default/files/media_file/2021-08/MLG-8.12.pdf

Hitchins, A.D., Jinneman, K. and Chen, Y., FDA, CFSAN. 2017. "Chapter 10 – Detection and Enumeration of *Listeria monocytogenes* in Foods." *Bacteriological Analytical Manual Online.* https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration

7.2.12 Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.12.1
Post Decontamination	Culture and Real-Time PCR	7.2.12.2

[1] See Appendix C for corresponding method usability tiers.

7.2.12.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier I).
- Water samples should be processed according to EPA Method 1200 (U.S. EPA 2012, Tier 1).
- All other environmental sample types should be processed according to procedures within the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Jyoti et al. 2011, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Jyoti et al. 2011 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jyoti et al. 2011). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of non-typhoidal *Salmonella*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2012. "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water." Washington, DC: U.S. EPA. EPA 817-R-12-004. https://www.epa.gov/sites/production/files/2015-08/documents/epa817r12004.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. "Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction." *Environmental Science and Technology*. 45(20): 8996-9002. http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.12.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006. Tier I).

- Water samples should be processed according to EPA Method 1200 (U.S. EPA 2012, Tier I)
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Method 1682 [U.S. EPA 2006, Tier I] or EPA Method 1200 [U.S. EPA 2012, Tier I]) and real-time PCR (Jyoti et al. 2011, Tier II)

Description of Method: Following appropriate sample processing (see Sample Processing procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective agars. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Jyoti et al. 2011 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jyoti et al. 2011). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of non-typhoidal *Salmonella*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2012. "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water." Washington, DC: U.S. EPA. EPA 817-R-12-004. https://www.epa.gov/sites/production/files/2015-08/documents/epa817r12004.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. "Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction." *Environmental Science and Technology*. 45(20): 8996-9002. http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.13 *Salmonella enterica* serovar Typhi (*S.* Typhi) [Typhoid fever] – BSL-2; BSL-3 for Aerosol Release

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.13.1
Post Decontamination	Culture and Real-Time PCR	7.2.13.2

[1] See Appendix C for corresponding method usability tiers.

7.2.13.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier I).
- Water samples should be processed according to the *Salmonella* Typhi Protocol (referred to as the EPA ST Protocol [U.S. EPA 2010, Tier I]).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (CDC Laboratory Assay, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (CDC Laboratory Assay or the EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Salmonella* Typhi. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (EPA ST Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/133.

https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=230138

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, Foodborne and Diarrheal Diseases Branch, CDC, Atlanta, GA. https://www.nemi.gov/methods/method_summary/10303/

7.2.13.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier I).
- Water samples should be processed according to the EPA ST Protocol (U.S. EPA 2010, Tier I)
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (EPA ST Protocol [U.S. EPA 2010, Tier I]) and real-time PCR (CDC Laboratory Assay, Tier I)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are inoculated into broth media, incubated for 24 hours, and then inoculated and plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (CDC Laboratory Assay or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Salmonella* Typhi. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (EPA ST Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/133. https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=230138

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, Foodborne and Diarrheal Diseases Branch, CDC, Atlanta, GA. https://www.nemi.gov/methods/method_summary/10303/

7.2.14 *Shigella* spp. [Shigellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.14.1
Post Decontamination	Culture and Real-Time PCR	7.2.14.2

[1] See Appendix C for corresponding method usability tiers.

7.2.14.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Standard Method 9260 E (APHA et al. 2017, Tier I).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Cunningham et al. 2010, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Cunningham et al. 2010 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham et al. 2010). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid

detection of *Shigella* spp. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces with Accuracy as High as That of Culture." *Journal of Clinical Microbiology*. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

APHA, AWWA and WEF. 2017. "Method 9260 Detection of Pathogenic Bacteria E: *Shigella*." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.14.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Standard Method 9260 E (APHA et al. 2017, Tier I).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Standard Method 9260 E [APHA et al. 2017, Tier I]) and real-time PCR (Cunningham et al. 2010, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Cunningham et al. 2010 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham et al. 2010). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Shigella* spp. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." *Journal of Clinical Microbiology*. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

APHA, AWWA and WEF. 2017. "Method 9260 Detection of Pathogenic Bacteria E: *Shigella*." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.15 *Staphylococcus aureus* – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.15.1
Post Decontamination	Culture and Real-Time PCR	7.2.15.2

[1] See Appendix C for corresponding method usability tiers.

7.2.15.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Li et al. 2015 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Chiang et al. 2007, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Chiang et al. 2007 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Chiang et al. 2007). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Staphylococcus aureus*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

Li, H., Xin, H., and Li, S. F. 2015. "Multiplex PMA−qPCR Assay with Internal Amplification Control for Simultaneous Detection of Viable *Legionella pneumophila*, *Salmonella typhimurium*, and *Staphylococcus aureus* in Environmental Waters." *Environmental Science & Technology*. 49(24): 14249-14256. https://pubmed.ncbi.nlm.nih.gov/26512952/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." *Journal of Food Protection*. 70(12): 2855-2859. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-70.12.2855

7.2.15.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to Li et al. 2015 (Tier II).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (Standard Method 9213 B: *Staphylococcus aureus* [APHA et al. 2017, Tier I]) and real-time PCR (Chiang et al. 2007, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Chiang et al. 2007 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Chiang et al. 2007). Use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Staphylococcus aureus*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

Li, H., Xin, H., and Li, S. F. 2015. "Multiplex PMA−qPCR Assay with Internal Amplification Control for Simultaneous Detection of Viable *Legionella pneumophila*, *Salmonella typhimurium*, and *Staphylococcus aureus* in Environmental Waters." *Environmental Science & Technology*. 49(24): 14249−14256. https://pubmed.ncbi.nlm.nih.gov/26512952/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." *Journal of Food Protection*. 70(12): 2855-2859. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-70.12.2855

APHA, AWWA and WEF. 2017. "Method 9213 B: *Staphylococcus aureus*." *Standard Methods for the Examination of Water and Wastewater*. 23rd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.16 *Vibrio cholerae* [Cholera] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.16.1
Post Decontamination	Culture and Real-Time PCR	7.2.16.2

[1] See Appendix C for corresponding method usability tiers.

7.2.16.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to the EPA *Vibrio cholerae* Protocol (referred to as the EPA VC Protocol [U.S. EPA 2010, Tier I]).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Real-time PCR (Blackstone et al. 2007, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), the target nucleic acid should be extracted, purified (Blackstone et al. 2007 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Blackstone et al. 2007). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Vibrio cholerae*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2010. "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (EPA VC Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/139. http://nepis.epa.gov/Adobe/PDF/P100978K.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." *Journal of Microbiological Methods*. 68(2): 254-259. http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.16.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- Water samples should be processed according to the EPA VC Protocol (U.S. EPA 2010, Tier I).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier III).

Note: Refer to the EPA YP Protocol (U.S. EPA 2016) for ultrafiltration of large volume water samples.

Analytical Technique: Culture (EPA VC Protocol [U.S. EPA 2010, Tier I]) and real-time PCR (Blackstone et al. 2007, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated into enrichment broth, incubated for 8 hours, and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Blackstone et al. 2007 or EPA YP Protocol, Section 10.5 [U.S. EPA 2010]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Blackstone et al. 2007). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Vibrio cholerae*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. October 2010. "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (EPA VC Protocol). Cincinnati, OH: U.S. EPA. EPA 600/R-10/139. http://nepis.epa.gov/Adobe/PDF/P100978K.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." *Journal of Microbiological Methods*. 68(2): 254-259. http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.17 *Yersinia pestis* [Plague] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.17.1
Post Decontamination	RV-PCR (Water Samples)	7.2.17.2
	Culture and Real-Time PCR	7.2.17.3

[1] See Appendix C for corresponding method usability tiers.

7.2.17.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier I).

Analytical Technique: Real-time PCR (EPA YP Protocol [U.S. EPA 2016, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (EPA YP Protocol, Section 9.6), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Yersinia pestis*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.17.2 Post Decontamination Sample Analyses (RV-PCR) ─ Water Samples

Note: Laboratories without RV-PCR capability should analyze samples according to the culture procedure provided in Section 7.2.17.3.

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing: Water samples should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier I).

Analytical Technique: RV-PCR (EPA YP Protocol [U.S. EPA 2016, Tier I])

Description of Method: The RV-PCR procedure serves as an alternative to the traditional culture-based methods for detection of viable pathogens. The RV-PCR procedure integrates high-throughput sample processing, short-incubation broth culture, and highly sensitive and specific real-time PCR assays to detect low concentrations of viable *Yersinia pestis*. Prior to analysis, samples are processed using multiple extraction and wash steps. After mixing the water sample with growth medium, an aliquot (Time 0 [T_0]) is removed and stored at 4°C. The remaining broth

is then incubated for 24 hours at 30°C. After the incubation, an aliquot is removed (Time Final [T_f]). Both T_0 and T_f aliquots then go through DNA extraction and purification followed by real-time PCR analysis. The cycle threshold (C_T) values for the T_0 and T_f aliquots are then compared. The difference in C_T values between the T_0 and T_f is used to detect viable *Yersinia pestis*. A change (decrease) in the PCR $C_T \geq 6$ represents a 2-log increased DNA concentration in the T_f aliquot relative to the T_0 aliquot, which in turn represents an increase in DNA as a result of the growth of viable *Yersinia pestis* in the sample during the incubation period. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Sources:
U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.17.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Soil samples should be processed according to EPA Method 1682 (U.S. EPA 2006, Tier III).
- All other environmental sample types should be processed according to the EPA YP Protocol (U.S. EPA 2016, Tier I).

Analytical Technique: Culture and real-time PCR (EPA YP Protocol [U.S. EPA 2016, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), samples can be inoculated into enrichment broth prior to plating or plated directly on non-selective media and incubated for a minimum of three days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Yersinia pestis*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (BMBL, 6th Edition [CDC 2020]). https://www.cdc.gov/labs/BMBL.html

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.3 Method Summaries for Viruses

Summaries for the analytical methods listed in Appendix C for analysis of viral pathogens are provided in Sections 7.3.1 through 7.3.10. Each summary contains a brief description of the analytical methods selected for each viral pathogen, and links to, or sources for, obtaining full versions of the methods. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

7.3.1 Adenoviruses: Enteric and Non-enteric (A-F) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.3.1.1
Post Decontamination	Tissue Culture and Real-Time PCR	7.3.1.2

[1] See Appendix C for corresponding method usability tiers.

7.3.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier II).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time PCR (Jothikumar et al. 2005, Tier II)

Description of Method: Following appropriate sample processing (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Jothikumar et al. 2005), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters. Use of real-time PCR directly on samples (e.g., no tissue culture component) allows for rapid detection of adenoviruses. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE.* 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F.R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure."

Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." *Applied and Environmental Microbiology*. 71(6): 3131-3136. http://aem.asm.org/content/71/6/3131.full.pdf+html

7.3.1.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier II).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (Boczek et al. 2016 or Green and Loewenstein 2005, Tier II) and real-time PCR (Jothikumar et al. 2005, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples should be cultured to assess viability (Boczek et al. 2016 or Green and Loewenstein 2005). For confirmation, target nucleic acid should be extracted, purified (Jothikumar et al. 2005), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." *Applied and Environmental Microbiology*. 71(6): 3131-3136. http://aem.asm.org/content/71/6/3131.full.pdf+html

Boczek, L.A., Rhodes, E.R., Cashdollar, J.L., Ryu, J., Popovici, J., Hoelle, J.M., Sivaganesan, M., Hayes, S.L., Rodgers, M.R. and Ryu, H. 2016. "Applicability of UV Resistant *Bacillus pumilus* Endospores as a Human Adenovirus Surrogate for Evaluating the Effectiveness of Virus Inactivation in Low-pressure UV Treatment Systems." *Journal of Microbiological Methods*. 122: 43-49. http://www.sciencedirect.com/science/article/pii/S0167701216300124

Green, M., and Loewenstein, P.M. 2005. "UNIT 14C.1 Human Adenoviruses: Propagation, Purification, Quantification, and Storage." *Current Protocols in Microbiology*. 00:C:14C.1.1-14C.1.19. http://onlinelibrary.wiley.com/doi/10.1002/9780471729259.mc14c01s00/abstract

7.3.2 Astroviruses – BSL not specified

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.2.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.2.2

[1] See Appendix C for corresponding method usability tiers.

7.3.2.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (Grimm et al. 2004, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Grimm et al. 2004), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time reverse transcription-PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of astroviruses. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate ribonuclease (RNAse) inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Grimm, A.C., Cashdollar, J.L., Williams, F.P. and Fout, G.S. 2004. "Development of an Astrovirus RT-PCR Detection Assay for Use With Conventional, Real-Time, and Integrated Cell Culture/RT-PCR." *Canadian Journal of Microbiology*. 50(4): 269-278. https://cdnsciencepub.com/doi/abs/10.1139/w04-012

7.3.2.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Integrated cell culture and real-time reverse transcription-PCR (Grimm et al. 2004, Tier II)

Description of Method: The method is a real-time reverse transcription-PCR procedure that can be integrated with cell culture (CaCo-2 cells) to enhance sensitivity. Following the appropriate sample processing procedure (see Sample Processing Procedures above), concentrated samples are analyzed directly or indirectly, after cell culture, by a two-step real-time reverse transcription-PCR (i.e., reverse transcription followed by real-time PCR) assay using astrovirus-specific primers, probes and assay parameters (Grimm et al. 2004). ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Grimm, A.C., Cashdollar, J.L., Williams, F.P. and Fout, G.S. 2004. "Development of an Astrovirus RT-PCR Detection Assay for Use With Conventional, Real-Time, and Integrated Cell Culture/RT-PCR." *Canadian Journal of Microbiology*. 50(4): 269-278. https://cdnsciencepub.com/doi/abs/10.1139/w04-012

7.3.3 Caliciviruses: Noroviruses – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.3.1
Post Decontamination	No method available to determine viable virus after decontamination	7.3.3.2

[1] See Appendix C for corresponding method usability tiers.

7.3.3.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (EPA Method 1615 [Fout et al. 2012, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (EPA Method 1615 [Fout et al. 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. Real-time instrument requirements for the ROX passive reference dye concentration should be verified.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.3.2 Post Decontamination Sample Analyses

No method available to determine viable virus after decontamination.

7.3.4 Caliciviruses: Sapovirus – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.4.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.4.2

[1] See Appendix C for corresponding method usability tiers.

7.3.4.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (Oka et al. 2006, Tier II)

Description of Method: The method is a TaqMan (Thermo Fisher Scientific, Waltham, MA, or equivalent)-based real-time reverse transcriptase PCR assay that can detect four of the five distinct sapovirus genogroups (GI–GV) using a multiplex assay. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Oka et al. 2006), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." *Journal of Medical Virology*. 78(10): 1347-1353. http://onlinelibrary.wiley.com/doi/10.1002/jmv.20699/abstract

7.3.4.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (Parwani et al. 1991, Tier II) and real-time reverse transcription-PCR (Oka et al. 2006, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples should be cultured using LL-PK cells supplemented with intestinal contents preparation (ICP) to assess viability (Parwani et al. 1991). For confirmation, target nucleic acid should be extracted, purified (Oka et al. 2006), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters. ***Note***:

Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. Culture procedure is for porcine sapovirus and may not be appropriate for all strains of sapoviruses.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." *Journal of Medical Virology*. 78(10): 1347-1353. http://onlinelibrary.wiley.com/doi/10.1002/jmv.20699/abstract

Parwani, A.V., Flynn, W.T., Gadfield, K.L and Saif L.J. 1991. "Serial Propagation of Porcine Enteric Calicivirus in a Continuous Cell Line. Effect of Medium Supplementation With Intestinal Contents or Enzymes." *Archives of Virology*. 120(1-2): 115-122. https://doi.org/10.1007/bf01310954

7.3.5 Coronaviruses: Severe Acute Respiratory Syndrome (SARS) -associated Human Coronavirus (SARS-CoV-2, SARS-CoV, and MERS-CoV) – BSL-2; BSL-3 for Propagation

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.5.1
Post Decontamination	Rapid Viability-Reverse Transcription-PCR	7.3.5.2
	Tissue Culture and Reverse Transcription-PCR	7.3.5.3

[1] See Appendix C for corresponding method usability tiers.

7.3.5.1 Site Characterization Sample Analyses (Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Shah et al. 2021 (Tier II).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (McMinn et al. 2021, Tier II)

Description of Method: The method describes a real-time reverse transcription-PCR procedure that can detect coronaviruses in wastewater and may be adapted for assessment of air, surface and soil samples. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (McMinn et al. 2021), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. For additional assays, refer to Lu et al. 2020.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Shah, S.R., Kane, S.R., Elsheikh, M. and Alfaro, T.M. 2021. "Development of a rapid viability RT-PCR (RV-RT-PCR) method to detect infectious SARS-CoV-2 from swabs." *Journal of Virological Methods*. 297: 114251. https://doi.org/10.1016/j.jviromet.2021.114251

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

McMinn, B.R., Korajkic, A., Kelleher, J., Herrmann, M.P., Pemberton, A.C., Ahmed, W., Villegas, E.N., and Oshima, K. 2021. "Development of a large volume concentration method for recovery of coronavirus from wastewater." *Science of the Total Environment*. 774: 145727. https://doi.org/10.1016/j.scitotenv.2021.145727

Lu, X., Wang, L., Sakthivel, S.K., Whitaker, B., Murray, J., Kamili, S., Lynch, B., Malapati, L., Burke, S.A., Harcourt, J., Tamin, A., Thornburg, N.J., Villanueva, J.M. and Lindstrom, S. 2020. "US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2." *Emerging Infectious Diseases*. 26(8): 1654-1665. https://doi.org/10.3201/eid2608.201246.

7.3.5.2 Post Decontamination Sample Analyses (Rapid Viability-Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Shah et al. 2021 (Tier II).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: RV-RT-PCR (Shah et al. 2021, Tier II)

Description of Method: The rapid viability-reverse transcription-PCR procedure is a combination of a cell-culture-based viral enrichment and virus-gene-specific reverse transcription-PCR-based analysis. The reverse transcription-PCR analysis of SARS-CoV-2 RNA is conducted on the same sample both before (Time 0 [T_0]) and after (Time Final [T_f]) enrichment of the virus in cell-culture to determine the C_T difference. The sample is split into two equal aliquots for T_0 and T_f, with each aliquot added to a well with adhered cell monolayer on separate 96-well plates. After the 1-2 hour infection period, viral suspensions are removed and the cell culture is washed with 0.1 mL of maintenance medium. After removing the wash media, 0.1 mL of fresh maintenance medium is added. The T_0 well/plate is then processed immediately for RNA extraction and RT-PCR analysis. The remaining time-point wells/plates are incubated at 37°C with 5% CO_2 to the desired endpoint and processed for RNA extraction and RT-PCR analysis. The C_T values for the T_0 and T_f aliquots are then compared. The difference in C_T values between the T_0 and T_f is used to detect infectious virus in the sample. A change (decrease) in the PCR $C_T \geq 6$ represents ~ 2-log or more increase in SARS-CoV-2 RNA following enrichment. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213: 65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Shah, S.R., Kane, S.R., Elsheikh, M. and Alfaro, T.M. 2021. "Development of a rapid viability RT-PCR (RV-RT-PCR) method to detect infectious SARS-CoV-2 from swabs." *Journal of Virological Methods*. 297: 114251. https://doi.org/10.1016/j.jviromet.2021.114251

7.3.5.3 Post Decontamination Sample Analyses (Tissue Culture and Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Shah et al. 2021 (Tier II).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (Pagat et al. 2007, Tier II) and reverse transcription-PCR (McMinn et al. 2021, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto Vero cell monolayers; the cells are examined for cytopathic effects (CPE) to assess viability (Pagat et al. 2007). For confirmation, target nucleic acid should be extracted, purified (McMinn et al. 2021), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. For additional assays, refer to Lu et al. 2020.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Shah, S.R., Kane, S.R., Elsheikh, M. and Alfaro, T.M. 2021. "Development of a rapid viability RT-PCR (RV-RT-PCR) method to detect infectious SARS-CoV-2 from swabs." *Journal of Virological Methods*. 297: 114251. https://doi.org/10.1016/j.jviromet.2021.114251

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F.R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct

detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

McMinn, B.R., Korajkic, A., Kelleher, J., Herrmann, M.P., Pemberton, A.C., Ahmed, W., Villegas, E.N., and Oshima, K. 2021. "Development of a large volume concentration method for recovery of coronavirus from wastewater." *Science of the Total Environment*. 774: 145727. https://doi.org/10.1016/j.scitotenv.2021.145727

Pagat, A., Seux-Goepfert, R., Lutsch, C., Lecouturier, V., Saluzzo, J. and Kusters, I.C. 2007. "Evaluation of SARS-Coronavirus Decontamination Procedures." *Applied Biosafety*. 12(2): 100-108. https://doi.org/10.1177/153567600701200206

Lu, X., Wang, L., Sakthivel, S.K., Whitaker, B., Murray, J., Kamili, S., Lynch, B., Malapati, L., Burke, S.A., Harcourt, J., Tamin, A., Thornburg, N.J., Villanueva, J.M. and Lindstrom, S. 2020. "US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2." *Emerging Infectious Diseases*. 26(8): 1654-1665. https://doi.org/10.3201/eid2608.201246.

7.3.6 Hepatitis E Virus (HEV) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.6.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.6.2

[1] See Appendix C for corresponding method usability tiers.

7.3.6.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015(Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol

(UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (Jothikumar et al. 2006, Tier II)

Description of Method: The method uses a TaqMan real-time reverse transcription-PCR assay using the R.A.P.I.D. PCR systems to detect and quantitate all four major HEV genotypes. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Jothikumar et al. 2006), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F.R., and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." *Journal of Virological Methods*. 131(1): 65-71. http://www.sciencedirect.com/science/article/pii/S0166093405002417?via%3Dihub

7.3.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (Zaki et al. 2009, Tier II) and real-time reverse transcription-PCR (Jothikumar et al. 2006, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto HPG11 cells; the cells are examined for CPEs to assess viability (Zaki et al. 2009). For confirmation, target nucleic acid should be extracted, purified (Jothikumar et al. 2006), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R., and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct

detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." *Journal of Virological Methods*. 131(1): 65-71. http://www.sciencedirect.com/science/article/pii/S0166093405002417?via%3Dihub

Zaki, M., Foud, M.F. and Mohamed, A. F. 2009. "Value of Hepatitis E Virus Detection by Cell Culture Compared With Nested PCR and Serological Studies by IgM and IgG." *Pathogens and Disease*. 56(1): 73-79. https://doi.org/10.1111/j.1574-695X.2009.00552.x

7.3.7 Influenza H5N1 virus – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.7.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.7.2

[1] See Appendix C for corresponding method usability tiers.

7.3.7.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier II).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Real-time reverse transcription-PCR (Ng et al. 2005, Tier II)

Description of Method: This is a two-step, real-time reverse transcriptase-PCR multiplex assay. The assay is specific for the H5 subtype. ***Note***: Influenza H5N1 virus samples are to be handled with BSL-3 containment and practices. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified

(Ng et al. 2005), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Ng, E.K.O., Cheng, P.K.C., Ng, A.Y.Y., Hoang, T.L. and Lim, W.W.L. 2005. "Influenza A H5N1 Detection." Emerging Infectious Diseases. 11(8): 1303-1305. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320469/

7.3.7.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier II).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Tissue culture (Krauss et al. 2012, Tier II) and real-time reverse transcription-PCR (Ng et al. 2005, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto Madin-Darby Canine Kidney Cells (MDCK); the cells are examined for CPEs to assess viability (Krauss et al. 2012). For confirmation, target nucleic acid should be extracted, purified (Ng et al. 2005), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure."

Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Ng, E.K.O., Cheng, P.K.C., Ng, A.Y.Y., Hoang, T.L. and Lim, W.W.L. 2005. "Influenza A H5N1 Detection." *Emerging Infectious Diseases*. 11(8): 1303-1305. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320469/

Krauss, S., Walker, D. and Webster, R.G. 2012. "Influenza Virus Isolation." *Methods in Molecular Biology*. 865: 11-24. https://www.ncbi.nlm.nih.gov/pubmed/22528151

7.3.8 Picornaviruses: Enteroviruses – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.8.1
Post Decontamination	Tissue Culture	7.3.8.2

[1] See Appendix C for corresponding method usability tiers.

7.3.8.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (EPA Method 1615 [Fout et al. 2012, Tier I])

Description of Method: The method uses a TaqMan real-time reverse transcriptase-PCR assay to detect and quantify enteroviruses. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Method 1615 [Fout et al. 2012]). ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. Real-time instrument requirements for the ROX passive reference dye concentration should be verified.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.8.2 Post Decontamination Sample Analyses (Tissue Culture)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol

(UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (EPA Method 1615 [Fout et al. 2012, Tier I])

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following the appropriate sample processing procedure (see Sample Processing Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days (EPA Method 1615 [Fout et al. 2012]). ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.9 Picornaviruses: Hepatitis A Virus (HAV) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.9.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.9.2

[1] See Appendix C for corresponding method usability tiers.

7.3.9.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (Hyeon et al. 2011, Tier II)

Description of Method: The method is a multiplex real-time reverse transcription-PCR procedure optimized for the simultaneous detection of enteroviruses, HAV, reoviruses and rotaviruses. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Hyeon et al. 2011), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Hyeon, J. Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method for Hepatitis A Virus from Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR." *Journal of Food Protection*. 74(10): 1756-1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.9.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Integrated cell culture and real-time reverse transcription-PCR (Hyeon et al. 2011, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are inoculated onto fetal rhesus monkey kidney (FRhK-4) cells, and the cells are examined for CPE to assess viability. For confirmation, target nucleic acid should be extracted, purified (Hyeon et al. 2011), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Hyeon, J.Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method for Hepatitis A Virus from Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR." *Journal of Food Protection*. 74(10): 1756-1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.10 Reoviruses: Rotavirus (Group A) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.10.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.10.2

[1] See Appendix C for corresponding method usability tiers.

7.3.10.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Real-time reverse transcription-PCR (Jothikumar et al. 2009, Tier II)

Description of Method: The method is used to detect rotavirus using a one-step real-time reverse-transcription PCR. Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Jothikumar et al. 2009), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F.R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." *Journal of Virological Methods*. 155(2): 126-131. http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.3.10.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to Raynor et al. 2021 (Tier III).
- Surface samples should be processed according to Park et al. 2015 (Tier III).
- Soil samples should be processed according to Staggemeier et al. 2015 (Tier III).
- Water samples should be processed according to Method 1642 (U.S. EPA 2018, Tier III) for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) for volumes ≥ 10 L.

Analytical Technique: Tissue culture (EPA Method 1615 [Fout et al. 2012, Tier III]) and real-time reverse transcription-PCR (Jothikumar et al. 2009, Tier II)

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following appropriate sample processing (see Sample Processing Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days (EPA Method 1615 [Fout et al. 2012]). For confirmation, target nucleic acid should be extracted, purified (Jothikumar et al. 2009), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Raynor, P.C., Adesina A., Aboubakr, H.A., Yang, M., Torremorell, M. and Goyal, S.M. 2021. "Comparison of samplers collecting airborne influenza viruses:1. Primarily impingers and cyclones." *PLoS ONE*. 16(1):e0244977. https://doi.org/10.1371/journal.pone.0244977

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." *Applied and Environmental Microbiology*. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Staggemeier, R., Bortoluzzi, M., Moraes da Silva Heck, T., da Silva, T., Spilki, F. R. and Esteves de Matos Almeida, S. 2015. "Molecular detection of human adenovirus in sediment using a direct detection method compared to the classical polyethylene glycol precipitation." *Journal of Virological Methods*. 213:65-67. https://pubmed.ncbi.nlm.nih.gov/25486079/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." *Journal of Virological Methods*. 155(2): 126-131. http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.4 Method Summaries for Protozoa

Summaries for the analytical methods listed in Appendix C for analysis of protozoa are provided in Sections 7.4.1 through 7.4.5. Each summary contains a brief description of the analytical methods selected for each protozoan, and links to, or sources for, obtaining full versions of the methods. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

7.4.1 *Cryptosporidium* spp. [Cryptosporidiosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.1.1
	IMS/immunofluorescence assay (FA)	7.4.1.2 [2]
	IMS/FA	7.4.1.3 [2]
Post Decontamination	Cell Culture Immunofluorescence Procedure	7.4.1.4

[1] See Appendix C for corresponding method usability tiers.

[2] Methods 1622 and 1623.1 include the same sample processing and analytical procedures for *Cryptosporidium*; either method could be used.

7.4.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Zopp et al. 2016 (Tier II).
- Water samples should be processed according to EPA Method 1622 (U.S. EPA 2005, Tier I), EPA Method 1623.1 (U.S. EPA 2012, Tier I), or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Real-time PCR (Guy et al. 2003 and Jiang et al. 2005, Tier II)

Description of Method: Following appropriate sample processing (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Guy et al. 2003, Jiang et al. 2005 or EPA BA Protocol, Section 9.2 [U.S. EPA 2017]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Guy et al. 2003). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Cryptosporidium* spp. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Zopp, Z.P, Olstadt, J. M., Karthikeyan, K.G., Thompson, A.M. and Long, S.C. 2016. "*Cryptosporidium* Soil Extraction by Filtration/IMS/FA Compatible with USEPA Method 1623.1" *Agriculture & Environmental Letters*. 1(1):160031. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/ael2016.08.0031

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

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7.4.1.2 Site Characterization Sample Analyses (Immunomagnetic Separation/Immunofluorescence Assay [IMS/FA])

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Zopp et al. 2016 (Tier II).
- Water samples should be processed according to EPA Method 1622 (U.S. EPA 2005, Tier I), Method 1623.1 (U.S. EPA 2012, Tier I), or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: IMS and FA microscopy (EPA Method 1622 [U.S. EPA 2005, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are centrifuged to pellet the oocysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts. The oocysts are stained on well slides with fluorescently labeled monoclonal antibodies (mAbs) and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts. This method is not intended to determine viability, species, or infectivity of the oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, matrix spike/matrix spike duplicate (MS/MSD) and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed as stipulated in the method.

Sources:
U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
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Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores from Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Zopp, Z.P, Olstadt, J. M., Karthikeyan, K.G., Thompson, A.M. and Long, S.C. 2016. "*Cryptosporidium* Soil Extraction by Filtration/IMS/FA Compatible with USEPA Method 1623.1" *Agriculture & Environmental Letters*. 1(1):160031. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/ael2016.08.0031

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

7.4.1.3 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Zopp et al. 2016 (Tier II).
- Water samples should be processed according to EPA Method 1622 (U.S. EPA 2005, Tier I), EPA Method 1623.1 (U.S. EPA 2012, Tier I), or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: IMS and FA microscopy (EPA Method 1623.1 [U.S. EPA 2012, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia*

antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Zopp, Z.P, Olstadt, J. M., Karthikeyan, K.G., Thompson, A.M. and Long, S.C. 2016. "*Cryptosporidium* Soil Extraction by Filtration/IMS/FA Compatible with USEPA Method 1623.1" *Agriculture & Environmental Letters*. 1(1):160031. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/ael2016.08.0031

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

7.4.1.4 Post Decontamination Sample Analyses (Cell Culture Immunofluorescence Procedure)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Zopp et al. 2016 (Tier II).
- Water samples should be processed according to EPA Method 1622 (U.S. EPA 2005, Tier I), EPA Method 1623.1 (U.S. EPA 2012, Tier I), or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Cell culture immunofluorescence procedure (Bukhari et al. 2007, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are used to inoculate HCT-8 monolayers and incubated. Following incubation, the monolayers are examined using immunofluorescence to determine the number of viable oocysts present in the sample. The use of cell culture immunofluorescence analyses is a cost effective and expedient alternative to mouse infectivity assays to determine in vitro infectivity of *Cryptosporidium* oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
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Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Zopp, Z.P, Olstadt, J. M., Karthikeyan, K.G., Thompson, A.M. and Long, S.C. 2016.

"*Cryptosporidium* Soil Extraction by Filtration/IMS/FA Compatible with USEPA Method 1623.1" *Agriculture & Environmental Letters*. 1(1):160031. https://acsess.onlinelibrary.wiley.com/doi/full/10.2134/ael2016.08.0031

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Bukhari, Z., Holt, D.M., Ware, M.W. and Schaefer III, F.W. 2007. "Blind Trials Evaluating In Vitro Infectivity of *Cryptosporidium* Oocysts Using Cell Culture Immunofluorescence." *Canadian Journal of Microbiology*. 53(5): 656–663. https://cdnsciencepub.com/doi/10.1139/W07-032

7.4.2 *Entamoeba histolytica* – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.2.1
Post Decontamination	Cell Culture	7.4.2.2

[1] See Appendix C for corresponding method usability tiers.

7.4.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Ogbolu et al. 2011 (Tier II).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Real-time PCR (Mejia et al. 2013, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Mejia et al. 2013 or EPA BA Protocol, Section 9.2 [U.S. EPA 2017]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Mejia et al. 2013). The use of real-time PCR analyses directly on samples allows for rapid detection of *Entamoeba histolytica*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Ogbolu, D.O., Alli, O.A., Amoo, A.O., Olaosun, I.I., Ilozavbie, G.W. and Olusoga-Ogbolu, F.F. 2011. "High-level parasitic contamination of soil sampled in Ibadan metropolis." *African Journal of Medicine and Medical Sciences*. 40(4):321-5. https://pubmed.ncbi.nlm.nih.gov/22783681/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Mejia, R., Vicuña, Y., Broncano, N., Sandoval, C., Vaca, M., Chico, M., Cooper, P.J. and Nutman, T.B. 2013. "A Novel, Multi-parallel, Real-time Polymerase Chain Reaction Approach for Eight Gastrointestinal Parasites Provides Improved Diagnostic Capabilities to Resource-limited At-risk Populations." The American Journal of Tropical Medicine and Hygiene. 88(6): 1041–1047. https://doi.org/10.4269/ajtmh.12-0726

7.4.2.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Ogbolu et al. 2011 (Tier II).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Cell culture (Stringert 1972, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), *Entamoeba histolytica* cysts are placed in a modified trypticase-panmede liver digest-serum medium and incubated for 10 hours. Live amoebae excyst through a rupture in the cyst wall, whereas non-viable amoebae remain encysted. Microscopic examination of an aliquot of the incubated excystation culture allows calculation of the percent of empty (live) cysts and full (dead) cysts in a population.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Ogbolu, D.O., Alli, O.A., Amoo, A.O., Olaosun, I.I., Ilozavbie, G.W. and Olusoga-Ogbolu, F.F. 2011. "High-level parasitic contamination of soil sampled in Ibadan metropolis." *African Journal of Medicine and Medical Sciences*. 40(4):321-5. https://pubmed.ncbi.nlm.nih.gov/22783681/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Stringert, R.P. 1972. "New Bioassay System for Evaluating Percent Survival of *Entamoeba histolytica* Cysts." *The Journal of Parasitology*. 58(2): 306-310. http://www.jstor.org/discover/10.2307/3278094?uid=3739704&uid=2129&uid=2&uid=70&uid=4&uid=3739256&sid=47698759181407

7.4.3 *Giardia* spp. [Giardiasis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.3.1
	IMS/FA	7.4.3.2
Post Decontamination	Cell Culture	7.4.3.3

7.4.3.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Liang and Keeley 2011 (Tier III).
- Water samples should be processed according to EPA Method 1623.1 (U.S. EPA 2012, Tier I) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Real-time PCR (Guy et al. 2003, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Guy et al. 2003 or the EPA BA Protocol [U.S. EPA 2017]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Guy et al. 2003). The use of real-time PCR analyses directly on samples allows for rapid detection of *Giardia*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this

protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." *Applied and Environmental Microbiology*. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Guy, R.A., Payment, P., Krull, U.J. and Horgen, P.A. 2003. "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage." *Applied and Environmental Microbiology*. 69(9): 5178-5185. http://aem.asm.org/content/69/9/5178.full.pdf+html

7.4.3.2 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Liang and Keeley 2011 (Tier III).
- Water samples should be processed according to EPA Method 1623.1 (U.S. EPA 2012, Tier I) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: IMS and FA microscopy (Method 1623.1 [U.S. EPA 2012, Tier I])

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." *Applied and Environmental Microbiology*. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

7.4.3.3 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Liang and Keeley 2011 (Tier III).
- Water samples should be processed according to EPA Method 1623.1 (U.S. EPA 2012, Tier I) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Cell culture (Keister 1983, Tier II)

Description of Method: Procedures are described for analysis of cell culture samples and may be adapted for assessment of air, surface, soil and water samples (see Sample Processing Procedures above). Trypticase-yeast-iron-serum medium supplemented with bovine bile and additional cysteine is used to isolate and culture *Giardia lamblia*. *G. lamblia* is incubated for intervals of 72 and 96 hours at 36°C in borosilicate glass tubes. The cells form a dense, adherent monolayer on the surface of the glass or are observed swimming through the liquid medium.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." *Applied and Environmental Microbiology*. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Keister, D. 1983. "Axenic Culture of *Giardia lamblia* in TYI-S-33 Medium Supplemented With Bile." *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 77(4): 487-488. http://www.sciencedirect.com/science/article/pii/0035920383901207

7.4.4 *Naegleria fowleri* [Naegleriasis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.4.1
Post Decontamination	Culture and Real-Time PCR	7.4.4.2

[1] See Appendix C for corresponding method usability tiers.

7.4.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Most likely not of concern in air. See special considerations below.
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Mull et al. 2013 (Tier II).
- Water samples should be processed according to Standard Method 9750 (APHA et al. 2021, Tier I) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Real-time PCR (Mull et al. 2013, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), samples are concentrated by centrifugation. The pellet is then resuspended and further concentrated using IMS. Target nucleic acid should be extracted, purified (Mull et al. 2013 or EPA BA Protocol, Section 9.2 [U.S. EPA 2017]) and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Mull et al. 2013). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Naegleria fowleri*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Naegleria fowleri* has not been shown to spread via water vapor or aerosol droplets (see CDC's webpage on *Naegleria fowleri* at https://www.cdc.gov/parasites/naegleria/infection-sources.html).

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Mull, B.J., Jothikumar, N. and Hill, V.R. 2013. "Improved Method for the Detection and Quantification of *Naegleria fowleri* in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR." *Journal of Parasitology Research*. Article ID 608367: 8 pages. https://www.hindawi.com/journals/jpr/2013/608367/

APHA, AWWA and WEF. 2021. "Method 9750 Detection of *Naegleria Fowleri* in Water (Proposed)." *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

7.4.4.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Most likely not of concern in air. See special considerations below.
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Mull et al. 2013 (Tier II).
- Water samples should be processed according to Standard Method 9750 (APHA et al. 2021, Tier I) or the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III).

Analytical Technique: Culture (Standard Method 9750 [APHA et al. 2021, Tier I]) and real-time PCR (Mull et al. 2013, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing procedures above), sample concentrates are vortexed and plated with *E. coli*. Plates are incubated for 5 to 7 days and examined for trophozoites and cysts every 1 to 2 days using an inverted microscope with phase contrast microscopy. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Mull et al. 2013 or EPA BA Protocol, Section 11.6 [U.S. EPA 2017]) and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Mull et al. 2013). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Naegleria fowleri*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Naegleria fowleri* has not been shown to spread via water vapor or aerosol droplets (see CDC's webpage on *Naegleria fowleri* at https://www.cdc.gov/parasites/naegleria/infection-sources.html).

Sources:
U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati,

OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Mull, B.J., Jothikumar, N. and Hill, V.R. 2013. "Improved Method for the Detection and Quantification of *Naegleria fowleri* in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR." *Journal of Parasitology Research*. Article ID 608367: 8 pages.
https://www.hindawi.com/journals/jpr/2013/608367/

APHA, AWWA and WEF. 2021. "Method 9750 Detection of *Naegleria Fowleri* in Water (Proposed)." *Standard Methods for the Examination of Water and Wastewater*. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

7.4.5 *Toxoplasma gondii* [Toxoplasmosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.5.1
Post Decontamination	Cell Culture	7.4.5.2

[1] See Appendix C for corresponding method usability tiers.

7.4.5.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Lass et al. 2020 (Tier II).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III), or the EPA BA Protocol (U.S. EPA 2017, Tier III).

- Soil samples should be processed according to Escotte-Binet et al. 2019 (Tier II).
- Water samples should be processed according to Villegas et al. 2010 (Tier II) or EPA Method 1623.1 (U.S. EPA 2012, Tier III).

Analytical Technique: Real-time PCR (Yang et al. 2009, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Yang et al. 2009 or EPA BA Protocol, Section 9.2 [U.S. 2017]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Yang et al. 2009). The use of real-time PCR analyses directly on samples allows for rapid detection of *Toxoplasma gondii*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Lass, A., Szostakowska, B., Korzeniewski, K. and Karanis, P. 2017. "The first detection of Toxoplasma gondii DNA in environmental air samples using gelatine filters, real-time PCR and loop-mediated isothermal (LAMP) assays: Qualitative and quantitative analysis." *Parasitology*. 144(13): 1791-1801. http://dx.doi.org/10.1017/S0031182017001172

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Escotte-Binet, S., Da Silva, A.M., Cancès, B., Aubert, D., Dubey, J., La Carbona, S., Villena, I. and Poulle, M.L. 2019. "A rapid and sensitive method to detect *Toxoplasma gondii* oocysts in soil samples." *Veterinary Parasitology*. 274: 108904. https://doi.org/10.1016/j.vetpar.2019.07.012

Villegas, E.N., Augustine, S.A., Villegas, L.F., Ware, M.W., See, M.J., Lindquist, H.D.A., Schaefer, III, F.W. and Dubey, J.P. 2010. "Using Quantitative Reverse Transcriptase PCR and Cell Culture Plaque Assays to Determine Resistance of *Toxoplasma gondii* Oocysts to Chemical Sanitizers." *Journal of Microbiological Methods*. 81(3): 219-225. http://www.sciencedirect.com/science/article/pii/S0167701210001107

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

Yang, W., Lindquist, H.D. A., Cama, V., Schaefer III, F.W., Villegas, E., Fayer, R., Lewis, E.J., Feng, Y. and Xiao, L. 2009. "Detection of *Toxoplasma gondii* Oocysts in Water Sample Concentrates by Real-Time PCR." *Applied and Environmental Microbiology*. 75(11): 3477-3483. http://aem.asm.org/content/75/11/3477.full.pdf+html

7.4.5.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to Lass et al. 2020 (Tier II).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Escotte-Binet et al. 2019 (Tier II).
- Water samples should be processed according to Villegas et al. 2010 (Tier II) or EPA Method 1623.1 (U.S. EPA 2017, Tier III).

Analytical Technique: Cell culture (Villegas et al. 2010, Tier II)

Description of Method: Samples are subjected to a series of mechanical and chemical digestion steps to release sporozoites from the *Toxoplasma gondii* oocysts and then inoculated onto confluent fibroblast monolayers. Inoculated monolayers are then incubated undisturbed for ten days to allow for plaque formation. After ten days, the monolayers are fixed, stained with crystal violet, and examined for plaque formation. The literature reference also includes a quantitative polymerase chain reaction (qPCR) procedure to determine viability of *Toxoplasma gondii* oocysts; however, it may not be appropriate depending on the type of disinfection used during remediation.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Lass, A., Szostakowska, B., Korzeniewski, K. and Karanis, P. 2017. "The first detection of Toxoplasma gondii DNA in environmental air samples using gelatine filters, real-time PCR and loop-mediated isothermal (LAMP) assays: Qualitative and quantitative analysis." *Parasitology*. 144(13): 1791-1801. http://dx.doi.org/10.1017/S0031182017001172

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Escotte-Binet, S., Da Silva, A.M., Cancès, B., Aubert, D., Dubey, J., La Carbona, S., Villena, I. and Poulle, M.L. 2019. "A rapid and sensitive method to detect *Toxoplasma gondii* oocysts in soil samples." *Veterinary Parasitology*. 274: 108904. https://doi.org/10.1016/j.vetpar.2019.07.012

Villegas, E.N., Augustine, S.A., Villegas, L.F., Ware, M.W., See, M. J., Lindquist, H.D.A., Schaefer, III, F.W. and Dubey, J.P. 2010. "Using Quantitative Reverse Transcriptase PCR and Cell Culture Plaque Assays to Determine Resistance of *Toxoplasma gondii* Oocysts to Chemical Sanitizers." *Journal of Microbiological Methods*. 81(3): 219-225. http://www.sciencedirect.com/science/article/pii/S0167701210001107

U.S. EPA. 2012. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

7.5 Method Summaries for Helminths

Summaries for the analytical methods listed in Appendix C for analysis of helminths are provided in Section 7.5.1. The section contains a brief description of the analytical methods selected, and links to, or sources for, obtaining full versions of the methods. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

7.5.1 *Baylisascaris procyonis* [Raccoon roundworm fever] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.5.1.1
Post Decontamination	Embryonation of Eggs and Microscopy	7.5.1.2

[1] See Appendix C for corresponding method usability tiers.

7.5.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample processing and analysis procedures as summarized below

Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Kazacos et al. 1983 (Tier II).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) or Gatcombe et al. 2010 (Tier II).

Analytical Technique: Real-time PCR (Gatcombe et al. 2010, Tier II)

Description of Method: Following the appropriate sample processing procedure (see Sample Processing Procedures above), the target nucleic acid should be extracted, purified (Gatcombe et al. 2010 or EPA BA Protocol, Section 9.2 [U.S. EPA 2017]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Gatcombe et al. 2010). The use of real-time PCR analyses directly on samples (e.g., no embryonation or microscopic examination) allows for rapid detection of *Baylisascaris procyonis*. ***Note***: Commercially available kits appropriate for the organism and sample type may be used for nucleic acid extraction and purification.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213.
https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Kazacos, K.R. 1983. "Improved method for recovering ascarid and other helminth eggs from soil associated with epizootics and during survey studies." *American Journal of Veterinary Research*. 44(5): 896-900. https://pubmed.ncbi.nlm.nih.gov/6683477/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Gatcombe, R.R., Jothikumar, N., Dangoudoubiyam, S., Kazacos, K.R. and Hill, V.R. 2010. "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples." *Parasitology Research*. 106: 499-504. https://doi.org/10.1007/s00436-009-1692-6

7.5.1.2 Post Decontamination Sample Analyses (Embryonation of Eggs and Microscopy)

Method: This method includes a combination of sample processing and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of the following environmental sample types: air, surface, soil and water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Processing:

- Air samples should be processed according to the EPA BA Protocol (U.S. EPA 2017. Tier III).
- Surface samples should be processed according to Hodges et al. 2010 (Tier III), Rose et al. 2011 (Tier III) or the EPA BA Protocol (U.S. EPA 2017, Tier III).
- Soil samples should be processed according to Kazacos et al. 1983 (Tier II).
- Water samples should be processed according to the EPA and CDC Joint Collection Protocol (UF, U.S. EPA and CDC 2022, Tier III) and Method 1642 (filter processing, U.S. EPA 2018, Tier III) or Gatcombe et al. 2010 (Tier II).

Analytical Technique: Microscopy and embryonation of eggs (U.S. EPA 2003, Tier II)

Description of Method: The protocol describes procedures for analysis of soil and wastewater samples. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particles, the soils in the screened portion are allowed to settle out, and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate. This flotation procedure yields a layer likely to contain *Ascaris* and other parasite eggs, if present in the sample. Small particulates are removed by a second screening on a small mesh size screen. The resulting concentrate is incubated until control helminth eggs are fully embryonated. The concentrate is then microscopically examined for the categories of helminth ova on a counting chamber.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2017. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Incident, Second Edition" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-17/213. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=338673

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." *Journal of Microbiological Methods*. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." *Applied Environmental Microbiology*. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Kazacos, K.R. 1983. "Improved method for recovering ascarid and other helminth eggs from soil associated with epizootics and during survey studies." *American Journal of Veterinary Research*. 44(5): 896-900. https://pubmed.ncbi.nlm.nih.gov/6683477/

U.S. EPA and CDC. 2022. "Protocol for Collection of Water Samples for Detection of Pathogens and Biothreat Agents" (EPA and CDC Joint Collection Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-21/280. For access to this document, consult the appropriate contact in Section 4.0.

U.S. EPA. 2018. "Method 1642: Male-specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF) and Single Agar Layer (SAL) Procedure." Washington, DC: U.S. EPA. EPA-821-R-18-001. https://www.epa.gov/sites/default/files/2018-09/documents/method_1642_draft_2018.pdf

Gatcombe, R.R., Jothikumar, N., Dangoudoubiyam, S., Kazacos, K.R. and Hill, V.R. 2010. "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples." *Parasitology Research*. 106:499-504. https://doi.org/10.1007/s00436-009-1692-6

U.S. EPA. 2003. "Appendix I: Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge." *U.S. EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attractions in Sewage Sludge*. Cincinnati, OH: U.S. EPA EPA/625/R-92/013. https://www.epa.gov/sites/production/files/2015-07/documents/epa-625-r-92-013.pdf

Section 8.0: Selected Biotoxin Methods

Section 8 and Appendix D provide summary information regarding methods to be used in analyzing environmental samples for biotoxin contaminants during remediation activities following a contamination incident. The information is sorted alphabetically by biotoxin. For the purposes of this document, biotoxins are defined as poisonous chemicals or group of related chemicals that are derived from plants or animals, and include those that can be artificially produced in sufficient quantities as to represent a substantial hazard. Procedures and methods have been selected for each biotoxin that may need to be identified and/or quantified during remediation. Analytical procedures are not currently available for all the biotoxin/sample type combinations included in this document, and ongoing research efforts include identification of additional methods, as well as development and testing of some of the procedures listed. If updates become available, information will be provided on the SAM website (https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam).

Please note: This section provides guidance for selecting biotoxin methods to facilitate data comparability when laboratories are faced with a large-scale environmental remediation crisis. Not all methods have been verified for the biotoxin/sample type combination listed in Appendix D, and method usability tiers have been assigned to indicate the fitness of each method for its intended use. Users should refer to the specified methods and reference citations provided throughout Section 8.2 to identify biotoxin/sample type combinations that have been verified, and should also consider the possibility of analytical interferences inherent to environmental sample types (e.g., sample composition, the presence and concentrations of additional or competing biotoxins or biotoxin variants. Any questions regarding this information should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix D provides the following information:

- **Analyte(s).** The biotoxin of interest.
- **Chemical Abstracts Service Registry Number (CAS RN) / Description.** A unique identifier for substances that provides an unambiguous way to identify a biotoxin or biotoxin isoform when there are many possible systematic, generic or trivial names, and/or a brief statement describing the biotoxin.
- **Analysis type.** Tests are either for presumptive identification, confirmatory identification or biological activity determination; test types are described below.
- **Analytical technique.** Type of analytical instrumentation or assay used to determine the quantity and identity of compounds or components in a sample.
- **Analytical method.** The recommended method or procedure, and the corresponding publisher.
- **Aerosol (air filter, filter cassette or liquid impinger).** The recommended method/procedure to measure the analyte of interest in air sample collection media.
- **Solid (soil, powder).** The recommended method/procedure to measure the analyte of interest in solid samples.
- **Particulate (swab, wipe, filter cassette).** The recommended method/procedure to measure the analyte of interest in particulate sample collection media.
- **Non-drinking water.** The recommended method/procedure to measure the analyte of interest in water samples other than drinking water.
- **Drinking water.** The recommended method/procedure to measure the analyte of interest in drinking water samples.

Depending on site- and incident-specific goals, a determination of whether contaminant concentrations are above pre-existing levels may be necessary. Such determinations could involve investigations of

background levels at potentially uncontaminated locations in close proximity to the site, using methods listed in Appendix D. Other means might include examination of historical information regarding contaminant occurrence. For example, periodic episodes of some of the biotoxins (such as microcystins [MC] and other algal biotoxins) have been detected and measured in surface waters throughout the United States by methods similar to those in Appendix D (see, for example, http://toxics.usgs.gov/highlights/algal_toxins/). When using historical data, knowledge of the analytical methods and techniques used would be necessary, particularly in terms of their similarity in performance and quality control (QC) to the methods listed in Appendix D.

The "analysis types" identified in Appendix D are intended to address: (1) the level of certainty of results needed and (2) the remediation phase during which analytical support is needed (e.g., site assessment, clearance). Many of the presumptive methods that have been selected are immunoassays, which can be adapted for large-scale sample analysis while maintaining an appropriate level of analytical certainty. Confirmatory methods are generally more time consuming and expensive, and are intended to provide results with a higher level of certainty than those provided by presumptive methods. Methods that address biological activity tend to be even more time consuming and expensive, and are intended to provide a high level of certainty in corroborating other assay results. Note that the use of the terms "presumptive" and "confirmatory" in this document is not intended to redefine or supersede the Laboratory Response Network's (LRN) or any other organization's use of these terms.

A tiered approach, or algorithm, can be used when implementing the analysis types, particularly when needed to address a large number of samples. For example, methods identified as presumptive are generally more rapid than confirmatory methods, and might be used during the initial stages of remediation to evaluate the extent of contamination. Presumptive methods also might be used to identify samples that should be analyzed using the more extensive confirmatory methods. Confirmatory methods should be considered for use when: (1) presumptive analysis indicates the presence of the biotoxin, (2) a smaller subset of samples requires analysis, or (3) as required for a tiered approach to remediation. Depending on the goals of the remediation phase, biological activity methods may be needed because biotoxins are sometimes detectable but inactive; therefore, these assays may also provide information about potential impact on human safety.

In some cases, mass spectrometry (MS)-based procedures have been selected for either presumptive or confirmatory analysis. Once a sample is prepared, these procedures, particularly in conjunction with isotopically-labelled standards, generally provide relatively rapid and unambiguous detection and quantification of targeted biotoxins or associated biomarkers (e.g., abrine and ricinine), high sample throughput, and better analytical sensitivities than other techniques. The development and application of MS-based methods for monitoring biotoxins in foods and animal tissue by various regulatory agencies provides potential applicability to SAM matrices; in some cases, the sample preparation techniques required for MS analyses including cleanup, concentration, and/or extraction also can be applied to environmental samples. Liquid chromatography (LC)-MS-MS instrumentation, sample preparation techniques, and the availability of isotopically-labelled internal standards are continuously evolving. Users of SAM should consider whether additional research has occurred to improve applicability of these procedures for analysis of the SAM biotoxins, as well as improvements regarding the availability of appropriate labeled standards.

SAM does not recommend the use of cell-based or whole animal toxicity assays for determination of biological activity. This decision is based on the unsuitability of these assays to support remediation efforts, particularly with respect to their general availability, sample throughput capacity, relative sensitivity for some biotoxins, qualitative results and cost. Use of these assays may be warranted, however, in situations where a limited number of samples require further evaluation (e.g., cell based assays in the case of an unstudied biotoxin/sample type or the use of the mouse bioassay for presumptive positive results of high profile botulinum neurotoxin (BoNT) samples). For small molecule biotoxins in Appendix D, the presence of intact compound structure is an indication of biological activity; therefore,

the confirmatory method listed for these biotoxins also serves as a measure of biological activity. Both biological availability (i.e., biotoxin accessibility to site of action) and activity are required to elicit toxicity, and some *in vitro* methods may not address both parameters. The points of contact listed in Section 4.0 should be consulted for additional information regarding use of cell-based or whole animal toxicity assays.

Numerous analytical techniques using a variety of instrumentation have been selected and are cited in Appendix D. It is recognized that advances in procedures for analysis of biotoxins are frequently reported in the literature, and commercially available equipment for these analyses is evolving rapidly. Accordingly, the individual techniques and methods listed in Appendix D are to be regarded as a starting point – the user is encouraged to consult the SAM website (https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam) for updates. The availability of critical reagents (e.g., antibodies) and reference standards required for the selected analytical methods might be limited. In cases where specific information regarding their availability is not provided in the methods listed throughout Section 8.2, biotoxin methods points of contact listed in Section 4.0 should be contacted for additional information.

Additional research on biotoxin contaminants is ongoing within the U.S. Environmental Protection Agency (EPA) and includes the impact of disinfectants and preservatives. The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection (e.g., pH adjustors, de-chlorinating agents) may affect analytical results. When present, the impact of these agents on method performance should be evaluated, if not previously determined. EPA's Center for Environmental Solutions and Emergency Response (CESER, formerly National Homeland Security Research Center [NHSRC]) continues to maintain sample collection information documents that are intended as companions to SAM. These sample collection documents provide information regarding sampling container/media, preservation, holding time, sample sizes and shipping, and are available at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

8.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given biotoxin, as well as recommendations for QC procedures.

For information on the properties of the biotoxins listed in Appendix D, refer to the additional resources listed below. There are other biotoxins that may be of interest in environmental samples, in addition to those listed in Appendix D. It is beyond the scope of this section and Appendix D to discuss every possible biotoxin or their degradation products. Some examples of additional biotoxins are included in the resources listed below; these resources also contain additional general information that may be of use to laboratories performing biotoxin analysis.

Additional resources:

- *Defense Against Toxin Weapons*, published by the U.S. Army Medical Research Institute of Infectious Diseases (https://www.usamriid.army.mil/education/defensetox.htm), contains information regarding sample collection, biotoxin analysis and identification, as well as decontamination and water treatment.
- The Centers for Disease Control and Prevention (CDC) has additional information regarding select agent biotoxins (https://www.selectagents.gov/sat/index.htm).
- CDC's "Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition" (https://www.cdc.gov/labs/BMBL.html) includes some biotoxins.
- INCHEM contains both chemical and toxicity information (http://www.inchem.org/).

- The Registry of Toxic Effects of Chemical Substances (RTECS) database, accessed via the National Institute for Occupational Safety and Health (NIOSH) website at http://www.cdc.gov/niosh/rtecs/default.html for toxicity information.
- The *Forensic Science and Communications Journal* published by the Laboratory Division of the Federal Bureau of Investigation (FBI), accessed via http://www.fbi.gov/about-us/lab/forensic-science-communications.
- The U.S. National Response Team publishes Quick Reference Guides on a number of biotoxins (https://www.nrt.org/Main/Resources.aspx?ResourceType=Hazards%20(Oil,%20Chemical,%20Radiological,%20etc)&&ResourceSection=2&Category=Biological).

8.1.1 Standard Operating Procedures for Identifying Biotoxin Methods

The fitness of a method for its intended use is related to data quality objectives (DQOs) for a particular remediation activity. The tiers below have been assigned to the methods selected for each biotoxin/sample type pair to indicate a level of method usability for the specific biotoxin and sample type for which it has been selected. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), and appropriate QC. The sample types reflect representative examples and are not necessarily inclusive of all sample types that might be encountered by laboratories following a contamination incident.

Tier I: The biotoxin and sample type are both targets of the method(s). Data are available for all aspects of method performance and QC measures supporting its use without modifications.

Tier II: The biotoxin is a target of the method, and the method has been evaluated by one or more laboratories. The sample type may or may not be a target of the method, and available data and/or information regarding sample preparation indicate that analyses of similar sample types were successful. However, additional testing and/or modifications may be needed.

Tier III: The sample type is not a target of the method, and no reliable data supporting the method's fitness for its intended use are available. Data suggest, however, that the method(s) may be applicable with significant modification.

To determine the appropriate method for analysis of an environmental sample, locate the biotoxin of concern in Appendix D: Selected Biotoxin Methods under the "Analyte(s)" column. After locating the biotoxin, continue across the table row and identify the appropriate analysis type. After an analysis type has been chosen, find the analytical technique (e.g., immunoassay) and analytical method applicable to the sample type of interest (aerosol, solid, particulate, drinking water or non-drinking water).

Once a procedure has been identified in Appendix D, the corresponding procedure summary can be found in Section 8.2. Section 8.2 follows the organization of Appendix D, with biotoxins listed in alphabetical order and method summaries provided for each analysis type. Where available, a direct link to the references cited or to a source to obtain the reference cited is provided with the method summary. For additional information on sample preparation procedures and methods available through consensus standards organizations, please use the reference contact information provided in **Table 8-1**.

Table 8-1. Sources of Biotoxin Methods

Name	Publisher	Reference
EPA Analytical Methods	EPA Office of Water (OW) EPA CESER (formerly NHSRC)	https://www.epa.gov/dwanalyticalmethods https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam

Name	Publisher	Reference
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
American Public Health Association (APHA) Press Compendium	American Public Health Association	http://www.apha.org
Analytical Biochemistry*	Elsevier	https://www.journals.elsevier.com/analytical-biochemistry
Analytical Chemistry*	American Chemical Society (ACS)	http://www.acs.org/
Analytical Methods*	Royal Society of Chemistry	http://www.rsc.org/journals-books-databases/about-journals/analytical-methods/
Applied and Environmental Microbiology*	American Society for Microbiology (ASM)	http://aem.asm.org/
Austin Immunology	Austin Publishing Group	https://austinpublishinggroup.com/austin-immunology/
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*	Mary Ann Libert, Inc.	https://www.liebertpub.com/loi/bsp
Canadian Journal of Microbiology*	Canadian Science Publishing	https://cdnsciencepub.com/loi/cjm
Environmental Science and Technology*	ACS Publications	http://pubs.acs.org/page/esthag/about.html
Food Research International*	Elsevier	http://www.sciencedirect.com/science/journal/09639969
Harmful Algae*	Elsevier	https://www.journals.elsevier.com/harmful-algae
Inland Waters*	Taylor and Francis	https://www.tandfonline.com/toc/tinw20/current
International Journal of Food Microbiology*	Science Direct	http://www.sciencedirect.com/science/journal/01681605
Journal of Agricultural and Food Chemistry*	ACS Publications	http://pubs.acs.org/journal/jafcau
Journal of AOAC International*	AOAC International	https://www.aoac.org/journal-of-aoac-international/
Journal of Chromatography A*	Elsevier	https://www.journals.elsevier.com/journal-of-chromatography-a/
Journal of Food Protection*	International Association for Food Protection	https://www.foodprotection.org/publications/journal-of-food-protection/
Journal of Pharmaceutical and Biomedical Analysis*	Elsevier	https://www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis
Journal of the Science of Food and Agriculture*	John Wiley And Sons Ltd.	https://onlinelibrary.wiley.com/page/journal/10970010/homepage/productinformation.html

Name	Publisher	Reference
Journal of Shellfish Research*	National Shellfisheries Association	http://www.bioone.org/toc/shre/current
Letters in Applied Microbiology	Wiley Online Library	https://sfamjournals.onlinelibrary.wiley.com/journal/1472765x
Rapid Communications in Mass Spectrometry*	John Wiley And Sons Ltd.	https://analyticalsciencejournals.onlinelibrary.wiley.com/journal/10970231
PLOS ONE	PLOS	https://journals.plos.org/plosone/
Toxicon*	Elsevier	http://www.journals.elsevier.com/toxicon/
Toxins*	Molecular Diversity Preservation International (MDPI)	https://www.mdpi.com/journal/toxins

* Subscription and/or purchase required.

8.1.2 General QC Guidelines for Biotoxin Methods

Public officials must have data of known and documented quality to accurately assess the activities that may be needed in remediating a site during and following emergency situations. Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating properly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC.[18] Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated, and on the need to support timely decisions. Various levels of QC may be required if the data are generated during presence/absence determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and DQOs should be derived based on those needs and applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening, minimal QC samples (e.g., blanks, replicates) and documentation might be required to ensure data quality. Sample analyses for environmental evaluation during site assessment through site clearance, such as those identified in this document, might require additional QC (e.g., demonstrations of method sensitivity, precision and accuracy). It is also important to consider that, during the course of remediation, the concentration of biotoxins and these interferences are expected to change, potentially affecting certain analytical systems. For example, some immunologically based approaches and mass spectrometer designs – due to the underlying chemistry and physics – can yield false negatives or inaccurately low results if the amount of biotoxins and/or interferences exceeds the test's design criteria. Procedural and QC steps (e.g., dilution, matrix spikes) should be applied to ensure appropriate method performance for concentrations in sample taken throughout the remediation, including higher initial concentrations than might normally be expected in environmental samples.

The following describes a minimum set of QC samples and procedures that should be conducted for all analyses. Method-specific QC requirements may be included in some of the procedures cited in this document, and will be referenced in any EPA methods that are developed to address specific analytes and sample types of concern. Individual methods, sampling and analysis protocols, or contractual statements of work should be consulted to determine any additional QC that may be needed. QC tests should be run as frequently as necessary to ensure the reliability of analytical results. In general, sufficient QC includes an initial demonstration of measurement system capability as well as ongoing assessments to ensure the continued reliability of the analytical results.

[18] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality.

Examples of sufficient QC for the **presumptive** tests listed in Appendix D include:

- Blanks (e.g., method blanks, matrix blanks, solvent blanks, calibration blanks, reagent blanks)
- Positive test samples / negative test samples
- Calibration check samples
- Use of test kits and reagents prior to expiration date
- Accurate temperature controls (sample and reagent storage)

Examples of sufficient QC for the **confirmatory** tests listed in Appendix D include:

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks
- Demonstration of measurement system suitability for intended use
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte/sample type/level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)
- Demonstration of continued measurement system reliability
  - Matrix spike/matrix spike duplicates (MS/MSDs) recovery and precision
  - QC samples (system accuracy and sensitivity at levels of concern)
  - Continuing calibration verification
  - Method blanks

Please note: The type and quantity of appropriate quality assurance (QA) and QC procedures that will be required are incident-specific and should be included in incident-specific documents (e.g., Quality Assurance Project Plan [QAPP], Sampling and Analysis Plan [SAP], laboratory Statement of Work [SOW], analytical methods). This documentation and/or Incident Command should be consulted regarding appropriate QA and QC procedures prior to sample analysis.

8.1.3 Safety and Waste Management

All appropriate safety precautions should be used during collection, processing, and analysis of environmental samples. Laboratories should have a documented health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures included in the plan. Many of the methods summarized or cited in Section 8.2 contain some specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods also provide information regarding waste management. Additional information may be found in the following resources:

- American Biological Safety Association, Risk Group Classifications for Infectious Agents. Available at: https://my.absa.org/Riskgroups.
- CDC. 2009. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 6th Edition. Available at: https://www.cdc.gov/labs/BMBL.html.
- Fleming, D.O. and Hunt, D.L. (editors). 2017. *Biological Safety: Principles and Practices*, 5th Ed. American Society for Microbiology (ASM) Press: Herndon, VA. Available at: https://www.wiley.com/en-us/Biological+Safety%3A+Principles+and+Practices%2C+5th+Edition-p-9781683673132.
- CDC – 42 CFR part 73. Select Agents and Toxins. Available at: https://www.ecfr.gov/current/title-42/chapter-I/subchapter-F/part-73?toc=1.
- Department of Transportation (DOT) – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training

Requirements. Available at: https://www.ecfr.gov/current/title-49/subtitle-B/chapter-I/subchapter-C/part-172?toc=1.

- EPA – 40 CFR part 260. Hazardous Waste Management System: General. Available at: http://www.ecfr.gov/. Available at: https://www.ecfr.gov/current/title-40/chapter-I/subchapter-I/part-260?toc=1.
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: https://www.ecfr.gov/cgi-bin/text-idx?node=pt40.29.270&rgn=div5.
- Occupational Safety and Health Administration (OSHA) – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. Available at: https://www.ecfr.gov/current/title-29/subtitle-B/chapter-XVII/part-1910/subpart-Z/section-1910.1450.
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response. Available at: https://www.ecfr.gov/current/title-29/subtitle-B/chapter-XVII/part-1910/subpart-H/section-1910.120.
- U.S. Department of Agriculture (USDA) – 9 CFR part 121. Possession, Use, and Transfer of Select Agents and Toxins. Available at: https://www.ecfr.gov/current/title-9/chapter-I/subchapter-E/part-121.
- The Electronic Code of Federal Regulations (e-CFR). Available at: http://www.ecfr.gov/.

8.2 Method Summaries

Summaries of the analytical methods for the biotoxins listed in Appendix D are provided in Sections 8.2.1 through 8.2.21. Each section contains a brief description of the analytical methods selected, and links to, or sources for, obtaining full versions of the methods. For information regarding sample collection considerations for samples to be analyzed by these methods, see the latest version of the SAM companion Sample Collection Information Document at: https://www.epa.gov/esam/sample-collection-information-documents-scids.

8.2.1 Abrin / Abrine

CAS RN (Abrin): 1393-62-0
CAS RN (Abrine): 526-31-8

Description (Abrin): Toxin found in the seeds of jequirity pea (or rosary pea) plants. Contains glycoproteins, and consists of deadenylase (25–32 kDa A chain) and lectin (35 kDa B chain); an agglutinin (A2B2) may be present in crude preparations.
Description (Abrine): Small molecule, indole alkaloid marker for abrin.

Selected Methods	Analysis Type	Analytical Technique	Section
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 2014. 12(1): 49-62	Presumptive	Lateral Flow Immunoassay (LFA)	8.2.1.1
EPA/600-R-13/022. 2013. Version 1.0	Presumptive	Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS)	8.2.1.2
Journal of Food Protection. 2008. 71(9): 1868-1874	Presumptive	Immunoassays (Enzyme-Linked Immunosorbent Assay [ELISA] and Electrochemiluminescence [ECL])	8.2.1.3
Analytical Chemistry. 2017. 89(21): 11719-11727	Confirmatory	LC-MS-MS	8.2.1.4
Analytical Biochemistry. 2008. 378: 87-89	Biological Activity	Enzyme activity	8.2.1.5

8.2.1.1 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: Abrin in buffer, aerosol filters, and food powders
Method Selected for: These procedures have been selected for presumptive analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than aerosol filters.

Description of Method: This assay involves a lateral flow immunochromatographic device that uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second band in the control window indicates that the test strip functioned properly. Two bands or colored lines (in the sample and control windows) are required for a positive result determination. To complete a test on a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the window of the test strip and can be read visually or with a reader.

The method source (below) describes a multicenter evaluation of the sensitivity, specificity, reproducibility, and limitations of the LFA for abrin that can be used as a rapid qualitative test to detect the presence of abrin in environmental samples. Samples analyzed in this study included various powders of food and non-food substances (prepared in buffer) and aerosol samples (filter extracts). Using the test strip and the manufacturer's recommended threshold, the estimated limit of detection (LOD) of the LFA is approximately 10ng/mL or 1.5ng/reaction, which is well below clinically relevant levels (median lethal dose [LD_{50}] 3.3mg/kg inhaled and 20mg/kg ingested). Because this assay does not discriminate between abrin and *Abrus precatorius* agglutinin-1 (APA-1), it can only be used as a qualitative screening assay when testing unknown samples.

Special Considerations: This LFA is listed as Tier I for presumptive analysis of abrin in solid and aerosol samples, and Tier II for presumptive analysis of abrin in other environmental sample types. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative. The incorporation of a serial dilution step in the sample protocol can eliminate such potential errors.

Source: Ramage, J.G., Prentice, K.W., Morse, S.A., Carter, A.J., Datta, S., Drumgoole, R., Gargis, S.R., Griffin-Thomas, L., Hastings, H.P., Masri, H.P., Reed, M.S., Sharma, S.K., Singh, A.K., Swaney, E., Swanson, T., Gauthier, C., Toney, D., Pohl, J., Shakamuri, P., Stuchlik, O., Elder, I.A., Estacio, P.L., Farber, E.A.E., Hojvat, S., Kellogg, R.B., Kovacs, G., Stanker, L., Weigel, L., Hodge, D.R. and Pillai, S.P. 2014. "Comprehensive Laboratory Evaluation of a Specific Lateral Flow Assay for the Presumptive Identification of Abrin in Suspicious White Powders and Environmental Samples." *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*. 12(1): 49-62. https://doi.org/10.1089/bsp.2013.0080

8.2.1.2 Presumptive Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Abrine in drinking water
Method Selected for: This method has been selected for presumptive analysis of abrin by abrine detection in aerosol, solid, particulate and water samples. Abrine, an alkaloid present in equal concentrations with abrin in rosary peas (*Abrus precatorius* L.), is found in crude preparations of abrin and may be an indicator of abrin contamination. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method involves solid-phase extraction (SPE) of samples, followed by analysis of the extracts for abrine by liquid chromatography and LC-MS-MS. Samples are combined with isotopically-labeled internal standards and sample extracts are concentrated to dryness under nitrogen and heat, then adjusted to a 100-µL volume in high performance liquid chromatography (HPLC)-grade water. Accuracy and precision data are provided for application of the method to reagent water, as well as finished ground water and surface waters containing residual chlorine and/or chloramine. The method has a detection limit (DL) of 0.06 µg/mL and a minimum reporting level (MRL) of 0.50 µg/mL for abrine. The stability of 50 µg/mL abrine was tested in ground water and surface water samples stored at 4°C for up to 28 days. Percent recoveries of abrine were significantly reduced in chlorine-containing samples after five hours. Percent recoveries for chlorine-containing samples at five hours ranged from 9 to 21; percent recoveries at 28 days ranged from 8 (± 0) to 19% (± 1). Abrine was much more stable in monochloramine-containing samples. Percent recoveries for monochloramine-containing samples at 5 days ranged from 103 (± 3) to 107 (± 3); percent recoveries at 28 days ranged from 90 (± 4) to 93 (± 5).

Special Considerations: This method is listed as Tier I for presumptive analysis of abrin (as abrine) in drinking water and Tier II for presumptive analysis of abrin (as abrine) in all other environmental sample types. Performance data were generated using preserved water samples. If

concentrations of abrine are unexpectedly low (or absent), additional QC steps may be needed. The biotoxin methods points of contact listed in Section 4.0 should be consulted for additional information regarding analysis of sample types other than drinking water. While abrine can be used to indicate the presence of abrin, it can also be found alone, which can limit the usefulness of this determination.

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha-Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Knaack, J.S., Pittman, C.T., Wooten, J.V., Jacob, J.T, Magnuson, M., Silvestri, E. and Johnson, R.C. 2013. "Stability of ricinine, abrine, and alpha-amanitin in finished tap water." *Analytical Methods*. 20(5): 5804-5811. https://doi.org/10.1039/C3AY40304A

8.2.1.3 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA and ECL-based immunoassay)

Method Developed for: Abrin in food
Method Selected for: These procedures have been selected for presumptive analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: These commercially available immunoassays are used for detecting abrin in various food products. The procedures use mouse monoclonal antibodies (mAbs) and rabbit-derived polyclonal antibodies prepared against a mixture of abrin isozymes for three separate ELISA and ECL-based assays. The three assays vary by use of antibody combination (e.g., assay configuration): (1) polyclonal (capture)/polyclonal (detection) ELISA, (2) polyclonal/monoclonal ELISA and (3) polyclonal/monoclonal ECL assay. The LODs, with purified Abrin C and various abrin extracts in buffer, are between 0.1 and 0.5 ng/mL for all three assays. The LOD for abrin spiked into food products ranged from 0.1 to 0.5 ng/mL, using the ECL assay. The LOD for abrin spiked into food products for the ELISA assays ranged between 0.5 and 10 ng/mL depending on the antibody combination. In all cases, the LODs were less than the concentration at which abrin has been shown to pose a health concern.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of abrin in aerosol, solid, particulate and water samples. Sample preparation procedures used for foods suggest that similar aqueous extraction procedures may be applicable to environmental samples. Crude preparations of abrin may also contain agglutinins that are unique to rosary peas and that can cross-react in the immunoassays. Addition of non-fat milk powder to the sample buffer may eliminate false-positive results (Dayan-Kenigsberg, J., Bertocchi, A. and Garber, E.A.E. 2008. "Rapid Detection of Ricin in Cosmetics and Elimination of Artifacts Associated With Wheat Lectin." *Journal of Immunological Methods*. 336(2): 251-254.)
http://www.sciencedirect.com/science/journal/00221759

Source: Garber, E.A.E., Walker, J.L. and O'Brien, T.W. 2008. "Detection of Abrin in Food Using Enzyme-Linked Immunosorbent Assay and Electrochemiluminescence Technologies." *Journal of Food Protection*. 71(9): 1868-1874.
http://jfoodprotection.org/doi/abs/10.4315/0362-028X-71.9.1868

8.2.1.4 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Abrin in food (ham), beverages (milk), clinical (plasma), soil and river water

Method Selected for: These procedures have been selected for confirmatory analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than soil and water.

Description of Method: The source reference describes procedures for immuno-extraction, trypsin digestion, and LC-MS-MS detection and quantification of abrin and its isoforms in various matrices. Samples are incubated with magnetic beads coated with multiple abrin-specific antibodies, thereby concentrating and extracting abrin and isoforms. On-bead trypsin digestion, using an ultrasonic bath for digestion enhancement, results in reproducible peptide recovery in 30 minutes. A panel of common and isoform-specific peptides are then monitored by multiplex LC-MS-MS through the parallel reaction monitoring mode on a quadrupole-Orbitrap high resolution mass spectrometer. Absolute quantification is accomplished by isotope dilution using stable isotope-labeled peptides. This method was demonstrated as being sensitive and reproducible with a calibration range of 5 to 500 ng/mL.

Special Considerations: These procedures are listed as Tier I for confirmatory analysis of abrin in solid and water samples and Tier II for confirmatory analysis of abrin in all other environmental sample types. Sample preparation procedures used for foods, beverages and environmental samples (soil and water) suggest that similar immuno-extraction procedures may be applicable to other environmental samples. The additional resource cited below provides additional discussion of sample preparation, even though it utilizes matrix-assisted laser desorption ionization-time-of-flight mass spectrometry-time of flight (MALDI-TOF)-MS for abrin analysis. The resource describes procedures for immunoaffinity-enrichment using magnetic beads coated with specific abrin antibodies, elution of abrin from the beads prior to trypsin digestion, and the subsequent MALDI-TOF-MS analysis of abrin peptides using labeled peptides for quantification. The lower limit of detection for MALDI-TOF-MS was established at 40 ng/mL in milk and apple juice, which is higher than the LC-MS-MS method.

Source: Hansbauer, E., Worbs, S., Volland, H., Simon, S., Junot, C., Fenaille, F., Dorner, B.G., and Becher. F. 2017. "Rapid Detection of Abrin Toxin and Its Isoforms in Complex Matrices by Immuno-Extraction and Quantitative High Resolution Targeted Mass Spectrometry." *Analytical Chemistry*. 89(21): 11719-11727. https://doi.org/10.1021/acs.analchem.7b03189

Additional Resource: Livet, S., Worbs, S., Volland, H., Simon, S., Dorner, M.B., Fenaille, F., Dorner, B.G., and Becher, F. 2021. "Development and Evaluation of an Immuno-MALDI-TOF Mass Spectrometry Approach for Quantification of the Abrin Toxin in Complex Food Matrices." *Toxins*. 13(1): 52. https://doi.org/10.3390/toxins13010052

8.2.1.5 Analysis of Biological Activity

Analytical Technique: Enzyme activity

Method Developed for: Jequirity seed (abrin) and castor bean (ricin) extracts in buffer.
Method Selected for: These procedures have been selected for biological activity analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This *in vitro* assay is a ribonucleic acid (RNA) N-glycosidase enzyme activity assay that can be used for the detection of purified abrin or abrin in jequirity seed extracts. The method can be applied to both abrin and ricin, due to the similarity in enzyme activities of the two toxins. Synthetic biotinylated RNA substrates with varied loop sequences are cleaved by abrin toxin and the RNA products are hybridized to ruthenylated-oligodeoxynucleotides to generate an ECL signal. Assays require incubation for 2 hours at 48°C. Commercially available ECL-based reagents and ribonuclease (RNAse) inactivators are used. Control experiments for the jequirity seed experiments demonstrate lack of non-specific cleavage for the assay. The undiluted jequirity seed extract was assayed, with a resultant 21.6 ± 0.6 mg/mL total protein and 3.7 ± 0.3 µg/mL equivalents of toxin. Dilutions were performed to determine

effective signal-to-background ratio and the linear range for calculation of toxin activity. Undiluted jequirity seed extract contained a calculated level of 740 ± 50 µg/mL ricin activity equivalents, which greatly exceeded the immunoassay-based value.

Special Considerations: These procedures are listed as Tier II for analysis of the biological activity of abrin in aerosol, solid, particulate and water samples. The enzyme activity assay does not test for cell binding; other cell binding assays are in development, but are not currently available. At this time, the mouse bioassay is the only readily available assay to test for both cell binding and enzymatic activity of the intact (whole) toxin.

Source: Keener, W.K., Rivera, V.R., Cho, C.R., Hale, M.L., Garber, E.A.E. and Poli, M.A. 2008. "Identification of the RNA N-glycosidase Activity of Ricin in Castor Bean Extracts by an Electrochemiluminescence-Based Assay." *Analytical Biochemistry*. 378(1): 87-89. https://doi.org/10.1016/j.ab.2008.03.019

8.2.2 Aflatoxin

CAS RN: 27261-02-5 (B1), 22040-96-6 (B2), 1385-95-1 (G1), 7241-98-7 (G2)
Considered Variants: Aflatoxins B1, B2, G1 and G2

Description: Toxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Composed of difuranocoumarin molecules; B-group aflatoxins (B1 and B2) have a cyclopentane ring, while the G-group aflatoxins (G1 and G2) contain a lactone ring.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Method 991.31	Presumptive	Immunoaffinity (column) purification / LC-FL (detection)	8.2.2.1
See summary in 8.2.2.2	Presumptive	Immunoassay (LFA)	8.2.2.2
See summary in 8.2.2.3	Presumptive	Immunoassay (ELISA)	8.2.2.3
Journal of Agricultural and Food Chemistry. 2017. 65(33): 7138-7152	Confirmatory	LC-MS-MS	8.2.2.4

8.2.2.1 Presumptive Analysis

Analytical Technique: Immunoaffinity column purification and LC-fluorescence detector (FL)

Method Developed for: Aflatoxins in corn, raw peanuts and peanut butter
Method Selected for: These procedures have been selected for presumptive analyses of aflatoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involves extraction of samples with methanol/water, followed by sample filtration, dilution with water, and application to a commercially available affinity column containing mAbs specific for aflatoxins B_1, B_2, G_1 and G_2. Antibody-bound aflatoxins are eluted from the column with methanol. Reaction with bromine solution and subsequent fluorescence measurement is performed for detection and quantitation of total aflatoxins. Post-column iodine derivatization and LC-FL are performed for quantitation of individual aflatoxins. Method performance was characterized using various commodities (e.g., corn) for aflatoxin levels ranging from 10 to 30 ng/g.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of aflatoxins in aerosol, solid, particulate and water samples. The method was originally designed for the analysis of aflatoxins (B_1, B_2, G_1 and G_2) in samples where cleanup was necessary to remove food components such as fats and proteins. Research results indicate that cleanup and analyte concentration in water samples can be accomplished using an immunoaffinity column

containing a mAbs specific for aflatoxin B_1, B_2, G_1 and G_2 serotypes (Paterson, 2007; see additional resource citation below). Additional research indicates that soil samples can be extracted overnight in water/ethyl acetate and the supernatant evaporated to dryness, reconstituted in methanol/water, then filtered and eluted through a mini-column packed with aluminum oxide (Accinelli, 2008; see additional resource citation below). The method notes that AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts, which uses derivatization and a multifunctional cleanup column, can be used as a complementary LC-FL procedure.

Source: AOAC International. 1994. "Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter." *Official Methods of Analysis of AOAC International*. 16th Edition. 4th Revision; Vol. II. http://www.aoac.org/

Additional Resources:

- AOAC International. 1998. AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts. *Official Methods of Analysis of AOAC International*, 16th Edition. 4th Revision, Vol. II. http://www.aoac.org/.
- Paterson, R. R. M., Kelly, J. and Gallagher, M. 2007. "Natural occurrence of aflatoxins and *Aspergillus flavus* (Link) in water." *Letters in Applied Microbiology*. 25: 435-436.
- Accinelli, C., Abbas, H. K., Zablotowsicz, R. M. and Wilkinson, J. R. 2008. "*Asperigjullus flavus* aflatoxin biosynthesis genes in soil." *Canadian Journal of Microbiology*. 54: 371-379.

8.2.2.2 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: Total aflatoxins in grain commodities
Method Selected for: These procedures have been selected for presumptive analysis of total aflatoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial assay is a lateral flow immunochromatographic device that uses a competitive immunoassay format for qualitative and quantitative determination of total aflatoxins. Samples are extracted with methanol (70% in water), filtered, diluted, and applied to the sample pad of the test strip. Two bands or colored lines (in the sample and control windows) are required for a positive result determination. Results of the LFA are determined by digital analysis of the test strips following sample application and a 10-minute incubation period. Vendor reported measurement ranges are 2–75 ppb for sample extracts and 10–375 ppb for diluted samples.

Special Considerations: The procedures described above are listed as Tier III for presumptive analysis of aflatoxins in aerosol, solid, particulate and water samples. The procedures have been adapted from a commercial kit and, at the time of publication, information regarding assay performance in environmental samples is not available. When available, updates will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam. Please note that mention of commercial products does not constitute the Agency's endorsement.

Source: Adapted from Eurofins Rapidust Analysis. https://cdnmedia.eurofins.com/corporate-eurofins/media/1035/rapidust_brochure_en.pdf. Consult the technical contacts listed in Section 4.0 for additional information regarding this commercial assay.

8.2.2.3 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Quantitative analysis of aflatoxin in nuts, grain and grain products
Method Selected for: These procedures have been selected for presumptive analysis of

aflatoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available immunoassay is a competitive ELISA used to detect and quantify aflatoxins in nuts, grain and grain products. Aflatoxins are extracted from a ground sample by blending or shaking with methanol/water. The extract is diluted with water, filtered and tested in the immunoassay. Aflatoxin-horseradish peroxidase (HRP) enzyme conjugate is pipetted into the test wells followed by calibrators or sample extracts, and aflatoxin antibody is then pipetted into the test wells to initiate the reaction. During the 10-minute incubation period, aflatoxins from the sample and aflatoxin-HRP enzyme conjugate compete for binding to the aflatoxin antibody which, in turn, binds to the test well. Following incubation, the contents of the well are removed and the wells are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the wells and any bound enzyme-toxin conjugate causes its conversion to a blue color. Following a 10-minute incubation, the reaction is stopped and amount of color in each well is read. The color is compared to the color of the calibrators and the aflatoxin concentration of the samples is derived. Semi-quantitative results can be derived by simple comparison of the sample absorbance to the absorbance of the calibrator wells. Samples containing less color than a calibrator have a concentration of aflatoxin greater than the concentration of the calibrator; samples containing more color than a calibrator have a concentration less than the concentration of the calibrator. Quantitative interpretation requires graphing the absorbances of the calibrators (X axis) versus the log of the calibrator concentration (Y axis) on semi-log graph paper. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator are reported as < 2 ppb or >100 ppb, respectively. This ELISA does not differentiate between various aflatoxins but detects their presence to differing degrees. The vendor-provided specificities for aflatoxins using this ELISA are as follows: aflatoxin B1 (100%), aflatoxin B2 (25%), aflatoxin G1 (25%), aflatoxin G2 (4%).

Special Considerations: These procedures are listed as Tier III for presumptive analysis of aflatoxins in aerosol, solid, particulate and water samples. The procedures have been adapted from a commercial kit and, at the time of publication, information regarding assay performance in environmental samples is not available. When available, updates will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam. Please note that mention of commercial products does not constitute the Agency's endorsement.

Source: Adapted from Eurofins/Abraxis users guide. https://abraxis.eurofins-technologies.com/media/10800/ug-21-084-rev-01-aflatoxin-elisa_53012b.pdf. Consult the technical contacts listed in Section 4.0 for additional information regarding this commercial assay.

8.2.2.4 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins (including aflatoxins, deoxynivalenol, fumonisin, ochratoxin A and zearalenone) in corn, peanut butter and wheat flour
Method Selected for: These procedures have been selected for confirmatory analyses of aflatoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes a collaborative laboratory study to evaluate an LC-MS-MS procedure using commercially available ^{13}C-labeled internal standards for the simultaneous detection and quantification of multiple mycotoxins. The method described can be used to detect and quantify mycotoxins including: aflatoxins; deoxynivalenol; fumonisins B1, B2, and B3; ochratoxin A; and zearalenone. Procedures for sample fortification, extraction, filtration and centrifugation are described in addition to LC-MS-MS conditions and parameters

for various platforms used by the laboratories participating in the study. The ranges of analytical performance for the six laboratories depended on LC-MS instrument conditions (column injection volume, flow rate, etc.). For example, the average recoveries of the participating laboratories were in the range of 90–110%, with repeatability relative standard deviation $(RSD)_r$ (within laboratory) < 10% and reproducibility RSD_R (among laboratories) < 15%. The ranges for the LOQs were: aflatoxin B1 (0.005−0.1 ng/mL), aflatoxin B2 (0.005−0.1 ng/mL), aflatoxin G1 (0.005−0.5 ng/mL), aflatoxin G2 (0.01−0.5 ng/mL).

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of aflatoxins in aerosol, solid, particulate and water samples. The sample preparation procedures described for food/feed (extraction with acetonitrile/water, centrifugation, and filtration) may be applicable to environmental samples.

Source: Zhang, K., Schaab, M.R., Southwood, G., Tor, E.R., Aston, L.S., Song, W., Eitzer, B., Majumdar, S., Lapainis, T., Mai, H., Tran, K., El-Demerdash, A., Vega, V., Cai, Y., Wong, J.W., Krynitsky, A.J. and Begley, T.H. 2017. "Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)." *Journal of Agricultural and Food Chemistry*. 65(33): 7138-7152. https://doi.org/10.1021/acs.jafc.6b04872

8.2.3 Amanitin

CAS RN: 23109-05-9 (α-amanitin), 21150-22-1 (β-amanitin) and 21150-23-2 (γ-amanitin)
Considered Variants: α-amanitin, β-amanitin and γ-amanitin

Description: Toxins produced by the poisonous death cap mushroom, *Amanita phalloides*. One of a group of thermostable bicyclic octapeptides.

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of Food Protection. 2005. 68(6): 1294–1301	Presumptive	Immunoassay (ELISA)	8.2.3.1
Toxins. 2020. 12(2): 123	Presumptive	Immunoassay (LFA)	8.2.3.2
EPA 600/R-13/022	Confirmatory	LC-MS-MS	8.2.3.3

8.2.3.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: α-Amanitin, ricin and T-2 mycotoxin in food and beverages
Method Selected for: These procedures have been selected for presumptive analysis of α-amanitin and T-2 toxin (see Section 8.2.19.1) in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: These commercially available ELISAs detect α-amanitin in a variety of food and beverage samples at levels below those shown to be a health concern. Solid food samples are extracted with sodium phosphate buffer, then diluted with phosphate-buffered saline. Liquid beverage samples are diluted in sodium phosphate buffer. Prepared samples are analyzed according to the kit manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 26 minutes of incubation at 37°C. α-Amanitin can be detected in food products at 1 µg/g. Background responses are noted for some samples, but at less than the equivalent of 0.5 µg/g for most samples.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of α-amanitin in aerosol, solid, particulate and water samples. Sample preparation procedures used for foods and beverages suggest that similar procedures may be applicable to environmental samples.

If appropriate concentrations are present, sample dilution can be used to minimize background interferences.

Source: Garber, E.A.E., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." *Journal of Food Protection*. 68(6): 1294-1301. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294?=

8.2.3.2 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: Detection of α-amanitin, β-amanitin and γ-amanitin in urine samples
Method Selected for: These procedures have been selected for presumptive analysis of α-amanitin, β-amanitin and γ-amanitin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available LFA is a competitive immunoassay format utilizing mAbs for detection of amanitins. The assay format is rapid (10 minutes), and qualitative results are determined either visually or by digital photographic analysis. Due to the competitive assay format, signal intensity decreases with increasing concentrations of amatoxins in the sample. In undiluted urine samples, cut-off concentrations due to signal extinction are 10 ng/mL for α- and γ-amanitin and 100 ng/mL for β-amanitin. LODs for α- and γ-amanitin are 0.3 ng/mL and 1 ng/mL for β-amanitin.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of amatoxins in aerosol, solid, particulate and water samples. Liquid samples may be tested directly while other environmental samples will require extraction prior to assay; however, further research is needed to verify efficacy. The additional resource cited below describes a simple and rapid aqueous extraction procedure for dried mushrooms that may be applicable to solid and particulate environmental samples.

Source: Bever, C.S., Swanson, K.D., Hamelin, E.I., Filigenzi, M.; Poppenga, R.H., Kaae, J., Cheng, L.W., and Stanker, L.H. 2020. "Rapid, Sensitive, and Accurate Point-of-Care Detection of Lethal Amatoxins in Urine." *Toxins*. 12(2): 123. https://doi.org/10.3390/toxins12020123

Additional Resource: Bever, C.S., Adams, C.A., Hnasko, R.M., Cheng, L.W., and Stanker, L.H. 2020. "Lateral flow immunoassay (LFIA) for the detection of lethal amatoxins from mushrooms." *PLOS ONE*. 15(4): e0231781. https://doi.org/10.1371/journal.pone.0231781

8.2.3.3 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of ricinine, abrine, and α-amanitin in drinking water
Method Selected for: This method has been selected for presumptive analysis of α-amanitin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for sample types other than drinking water.

Description of Method: An isotope dilution LC-MS-MS method is used for the determination of α-amanitin in drinking water. Sample aliquots are combined with mixture containing an internal quantification standard for α-amanitin, pipetted into a 96-well SPE plate, and extracted. Extracts are concentrated to dryness under nitrogen and heat, adjusted to 100 µL with HPLC-grade water, and injected onto an HPLC-MS-MS operated in multiple reaction monitoring (MRM) mode. α-Amanitin is identified by comparing the acquired mass spectra, including ion ratios and retention times, to reference spectra and retention times for calibration standards acquired under identical HPLC-MS-MS conditions. Quantitation is performed using the internal standard technique.

Special Considerations: These procedures are listed as Tier I for confirmatory analysis of α-amanitin in drinking water and Tier II for confirmatory analysis of α-amanitin in all other environmental sample types. Extraction of non-aqueous samples prior to SPE may be required (Kaya, 2013). These procedures might be modified for application to soil, aerosol and particulate samples. Isotopically labeled α-amanitin internal standards were not available during method development, but efforts to synthesize these standards are in progress. The biotoxin contacts listed in Section 4.0 should be consulted for current status and availability of α-amanitin internal standards.

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Kaya, E., Yilmaz, I., Sinirlioglu, Z.A., Karahan, S., Bayram, R., Yaykasli, K.O., Colakoglu, S., Saritas, A. and Severoglu, Z. 2013. "Amanitin and pallotoxin concentration in *Amanita phalloides var. alba* mushroom." *Toxicon*. 76: 225-233.
http://www.sciencedirect.com/science/article/pii/S0041010113003942

8.2.4 Anatoxin-a

CAS RN: 64285-06-9
Considered Variants: NA

Description: Tropane-related bicyclic alkaloid produced by a variety of freshwater cyanobacteria species.

Selected Methods	Analysis Type	Analytical Technique	Section
Inland Waters. 2020. 10(1): 109-117	Presumptive	Immunoassay (ELISA)	8.2.4.1
EPA Method 545	Confirmatory	LC-MS-MS	8.2.4.2
EPA/600/R-17/130	Confirmatory	LC-MS-MS	8.2.4.3

8.2.4.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Anatoxin-a in non-drinking water
Method Selected for: This immunoassay procedure has been selected for presumptive analysis of anatoxin-a in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: This commercially available immunoassay is a direct competitive ELISA based on the recognition of anatoxin-a by mAbs. When present in a sample, anatoxin-a and an anatoxin-a-enzyme conjugate compete for the binding sites of mouse anti-anatoxin-a antibodies in solution. The anatoxin-a antibodies are then bound by a second antibody (anti-mouse) immobilized on the microtiter plate. After a wash step and addition of the substrate solution, a color signal is generated. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The intensity of the blue color is inversely proportional to the concentration of anatoxin-a present in the sample. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. A DL of approximately 0.1 µg/L anatoxin-a is reported by the manufacturer of this kit.

Special Considerations: This assay is listed as Tier I for presumptive analysis of anatoxin-a in

water samples and Tier II for aerosol, solid and particulate samples. Anatoxin-a will degrade when exposed to natural and artificial light and/or high pH conditions. Samples that have been exposed to natural or artificial light and/or treated with reagents that raise the natural sample pH may produce results that are falsely low. Sodium thiosulfate should not be used to treat drinking water samples, as it will degrade anatoxin-a, producing inaccurate (falsely low) results. No matrix effects have been observed with samples that have been treated with ascorbic acid at concentrations ≤ 1 mg/mL. Anatoxin-a is an intracellular, as well as extracellular, toxin. Therefore, to measure total anatoxin-a, a cell lysing procedure (e.g., three freeze-thaw cycles) will be required for samples containing intact cells.

Source: Graham, J.L., Dubrovsky, N.M., Foster, G.M.. King, L R., Loftin, K.A., Rosen, B.H. and Stelzer, E.A. 2020. "Cyanotoxin occurrence in large rivers of the United States." *Inland Waters*. 10(1): 109-117. https://doi.org/10.1080/20442041.2019.1700749

8.2.4.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Anatoxin-a in potable water
Method Selected for: This method has been selected for confirmatory analysis of anatoxin-a in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method is used to detect anatoxin-a in drinking water samples. Samples are frozen and thawed three times, then filtered. A 1-mL sample aliquot from the supernatant is combined with internal standards and analyzed using LC-MS-MS with ESI. Anatoxin-a is identified by comparing retention times and signals produced by unique mass to those of procedural calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports a lowest concentration minimum reporting level (LCMRL) of 0.018 µg/L for anatoxin-a in fortified reagent water. The working range reported in the method is 0.029–5.87 µg/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of anatoxin-a in drinking water, and Tier II for confirmatory analysis of anatoxin-a in aerosol, solid and particulate samples. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins. It may be possible to analyze relatively clean water samples by direct injection into the LC-MS-MS. Extraction of anatoxin-a from cyanobacterial biocrusts has been reported (Chrapusta, 2015). An additional resource below (Haddad *et al.* 2019) describes liquid-liquid extraction and SPE cleanup procedures for fish tissue that may facilitate preparation of non-aqueous environmental samples. These procedures might be modified for application to soil, aerosol and particulate samples, for analysis using the LC-MS-MS conditions described in EPA Method 545.

Source: U.S. EPA. April 2015. Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). Cincinnati, OH: U.S. EPA. EPA 815-R-15-009**.**
https://www.epa.gov/sites/default/files/2017-10/documents/epa_815-r-15-009_method_545.pdf

Additional Resources:

- Chrapusta, E., Wegrzyn, M., Zabaglo, K., Kaminski, A., Adamski, M., Wietrzyk, P. and Bialczyk, J. 2015. "Microcystins and anatoxin-a in Arctic biocrust cyanobacterial communities." *Toxicon*. 101: 35-40. http://www.sciencedirect.com/science/article/pii/S0041010115001130
- Haddad S.P., Bobbitt J.M., Taylor R.B., Lovin, L.M., Conkle, J.L., Chambliss, C.K. and

Brooks, B.W. 2019. "Determination of microcystins, nodularin, anatoxin-a, cylindrospermopsin, and saxitoxin in water and fish tissue using isotope dilution liquid chromatography tandem mass spectrometry." *Journal of Chromatography A*. 1599:66-74. https://doi.org/10.1016/j.chroma.2019.03.066

8.2.4.3 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Cylindrospermopsin and anatoxin-a in ambient freshwaters
Method Selected for: This method has been selected for confirmatory analysis of anatoxin-a in non-drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than untreated freshwater.

Description of Method: This method uses LC-MS-MS for the determination of anatoxin-a (combined intracellular and extracellular) in ambient freshwater. Samples are subjected to three freeze/thaw cycles, an internal standard is added, and the sample is filtered. Samples with significant cell densities may require centrifugation prior to filtration. An aliquot of the sample filtrate is injected into an LC equipped with an analytical column that is interfaced to an MS-MS capable of positive ion electrospray ionization (ESI). The analytes are separated and identified by comparing retention times and signals produced by unique mass transitions to retention times and mass transitions for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports a LCMRL of 0.097 µg/L and calculated DL of 0.049 µg/L for anatoxin-a in fortified reagent water.

Special Considerations: This method is listed as Tier I for confirmatory analysis of anatoxin-a in non-drinking water samples.

Source: U.S. EPA. November 2017. Determination of Cylindrospermopsin and Anatoxin-a in Ambient Freshwaters by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). Cincinnati, OH: U.S. EPA. EPA/600/R-17/130. https://www.epa.gov/water-research/single-laboratory-validated-method-determination-cylindrospermopsin-and-anatoxin

8.2.5 Botulinum Neurotoxins (BoNTs)

CAS RN: 93384-43-1 (BoNT-A), 93384-44-2 (BoNT-B), 93384-45-3 (BoNT-C), 93384-46-4 (BoNT-D), 93384-47-5 (BoNT-E), 107231-15-2 (BoNT-F), 107231-16-3 (BoNT-G)
Considered Variants: A–G

Description: Protein neurotoxin produced by *Clostridium botulinum* and related species. Composed of ~100 kDa heavy chain and ~50 kDa light chain; can be complexed with hemagglutinin and non-hemagglutinin components for total molecular weight (MW) of ~900 kDa.

Selected Methods	Analysis Type	Analytical Technique	Section
EPA Environmental Technology Verification (ETV) Program Reports	Presumptive	Immunoassay (LFA)	8.2.5.1
Analytical Biochemistry. 2011. 411 (2): 200-209	Presumptive	Immunocapture-Forster Resonance Energy Transfer (FRET)-based activity assay	8.2.5.2
U.S. Department of Homeland Security (DHS) Report	Presumptive	Immunoassay (fluorescent bead-based)	8.2.5.3

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of the Science of Food and Agriculture. 2014. 94: 707–712	Presumptive	Immunoassay (ECL)	8.2.5.4
Toxins. 2018. 10(11): 476	Presumptive	Immunoassay (B-cell Based)	8.2.5.5
Journal of Agricultural and Food Chemistry. 2015. 63(4): 1133-1141	Confirmatory	LC-MS-MS Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF-MS)	8.2.5.6
APHA Press Compendium of Methods, Chapter 32	Biological Activity	Mouse bioassay	8.2.5.7

8.2.5.1 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: BoNTs (Types A, B) in buffer or water samples
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: The commercial test strip is a lateral flow immunochromatographic device that uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second band in the control window indicates that the test strip functioned properly. Two bands or colored lines (in the sample and control windows) are required for a positive result determination. For a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and is read visually or with a reader.

The LFA kits have been evaluated by the EPA ETV Program for the detection of BoNTs Types A and B in concentrated (ultrafiltration [UF]) and unconcentrated drinking water. The reference source reports the lowest detectable concentration of BoNT Type A as 0.01 mg/L and Type B as 0.05 mg/L, with no false negatives detected when interferents are present. Reports and information associated with these evaluations are available at: https://www.epa.gov/sites/default/files/2015-07/documents/etv-biothreat092104.pdf.

Special Considerations: This assay is listed as Tier I for presumptive analysis of BoNTs in drinking water samples and Tier II for presumptive analysis of BoNTs in other environmental sample types. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative. The incorporation of a serial dilution step can eliminate such potential errors.

Source: Environmental Technology Verification Report. 2004. Anthrax, Botulinum Toxin, and Ricin Immunoassay Test Strips; available at: https://www.epa.gov/sites/default/files/2015-07/documents/etv-biothreat092104.pdf.

8.2.5.2 Presumptive Analysis

Analytical Technique: Immunocapture FRET-based activity assay

Method Developed for: BoNTs Serotypes A, B, D, E, F and G
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial FRET-based assay detects BoNT serotypes A and E, and serotypes B, D, F and G, using separate kits for each serotype group. The assays measure the ability of BoNTs to proteolytically cleave synthetic substrates that mimic the natural BoNT substrates (SNAP25 or VAMP2) in a sensitive, FRET-based format using most standard fluorescent plate readers. The substrates used in the assay encompass both the exosite binding sites and cleavage site of BoNT, resulting in high BoNT affinity for the substrate with femtomolar to picomolar detection sensitivities within a few minutes to a few hours. The FRET-based nature of the assays allows for real-time detection of BoNT proteolytic activity, enabling the determination of kinetic constants and enzymatic activity.

Special Considerations: This assay is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Application of the assay to complex or dilute sample types may require a preliminary antibody capture/enrichment procedure using magnetic beads conjugated to serotype-specific antibodies. Non-liquid samples such as soils, powders and aerosol filters will require an aqueous extraction step prior to antibody capture. The FRET-based detection assay and antibody-coated beads are both commercially available.

Source: Ruge, D.R., Dunning, F.M., Piazza, T.M., Molles, B.E., Adler, M., Zeytin, F.N. and Tucker, W.C. 2011. "Detection of six serotypes of BoNT using fluorogenic reporters." *Analytical Biochemistry*. 411: 200-209.
https://www.ncbi.nlm.nih.gov/pubmed/21216216

8.2.5.3 Presumptive Analysis

Analytical Technique: Immunoassay (fluorescent bead-based)

Method Developed for: BoNT Serotypes A, B, C, D, E, F and G
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This multiplexed immunoassay is based on a commercially available technology that uses antibody-coated fluorescent polystyrene microspheres or beads as an immunoassay reaction surface. The beads are optically encoded internally with two spectrally distinct fluorescent dyes which identify each of the beads. Using a fluidics system, individual beads pass by a red laser that identifies each bead set based on their unique internal dye signatures and hence, the antigen specificity assigned to each bead set. A green laser is used to detect a third spectrally distinct fluorescent dye that quantifies the extent of antibody-antigen reactions on the surface of each bead. Distinct bead sets, coupled with unique capture antibodies with specific reactivity to each of the seven BoNT Serotypes (A–G), are added as a mixture to an unknown sample. As specific beads contact specific antigenic components in the sample, the BoNTs are captured on the surface of the beads. The mixture is washed to remove unbound sample; then a biotin-labeled detection antibody is added and allowed to bind to the complex, followed by addition of fluorescent reporter streptavidin phycoerythrin (SA-PE). The fluorescent intensity of the reporter as read by the fluorescence reader is proportional to the amount of toxin bound to the bead. LODs ranged from 20 to 200 pg/mL for each serotype, which is similar to the definition of 1 mouse LD_{50} by the mouse bioassay.

Special Considerations: This method is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction and clarification (e.g., centrifugation) prior to assay.

Source: DHS. 2015. Multi-agency Report. Rapid Botulinum Toxin Assay Test, Evaluation and Validation Study Report. ***Note****:* This document is available only to select government agencies. Please consult technical contacts listed in Section 4.0 for additional information regarding this report.

8.2.5.4 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)

Method Developed for: BoNT-A in milk products
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial imaging-based ECL immunoassay detects BoNT-A. The imaging-based ECL detection method is a sandwich-format immunoassay that uses a target-specific immobilized capture antibody and a biotinylated detection antibody. The ECL assay uses 96-well multiarray assay plates with integrated screen-printed carbon electrodes. The electrodes act both as solid phase supports to capture reagents used in the solid phase binding assays, and as the source of electrical energy for inducing ECL. The basis of the ECL measurement is the ability of these labels to emit light when oxidized at an electrode surface in the presence of tertiary amine (i.e., tripropylamine). During the ECL measurement, the plate reader applies a voltage to electrodes in the wells of the multiarray plates and measures the resulting ECL from labeled detector antibodies incorporated in sandwich complexes on each spot in the well with a cooled charge-coupled device (CCD) sensor. The LOD of this ECL assay is 40 pg/mL for BoNT-A complex. The additional resource listed below (Cheng, L.W. and Stanker, L.H. 2013) describes the use of a similar ECL immunoassay for detection of both BoNT-A and BoNT-B in different liquids, liquified solid foods, and horse serum.

Special Considerations: This method is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Sample preparation procedures described in both the source and additional resource citations suggest that similar aqueous extraction may be applicable to environmental samples. Soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: Sachdeva, A., Singh, A.K. and Sharma, S.K. 2014. "An electrochemiluminescence assay for the detection of bio threat agents in selected food matrices and in the screening of *Clostridium botulinum* outbreak strains associated with type A botulism." *Journal of the Science of Food and Agriculture*. 94: 707-712.
http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6310/abstract

Additional Resource: Cheng, L.W. and Stanker, L.H. 2013. "Detection of Botulinum Neurotoxin Serotypes A and B Using a Chemiluminescent versus Electrochemiluminescent Immunoassay in Food and Serum." *Journal of Agricultural and Food Chemistry*. 61: 755-760.
https://www.ncbi.nlm.nih.gov/pubmed/23265581

8.2.5.5 Presumptive Analysis

Analytical Technique: Immunoassay (B-cell based)

Method Developed for: BoNT Serotype A in food and beverages

Method Selected for: These procedures have been selected for presumptive analysis of BoNT-A in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial biosensor assay relies on B-cells expressing antibodies specific for BoNT-A and an intracellular calcium-sensitive bioluminescent protein (aequorin) for qualitative determination of BoNT-A holotoxin in various food and beverage matrices. The BoNT-A assay requires multiple steps: (1) binding of sample antigens to magnetic immuno-capture beads, (2) recognition of the bead-bound antigens by the B-cell specific surface antibodies, and (3) signal transduction and light emission. Samples are incubated (30 minutes at room temperature) with magnetic beads coated with anti-BoNT-A antibodies to allow for the toxin:immunomagnetic bead complex to form a multi-valent epitope. B-cells that express membrane-bound antibodies that are specific to a different epitope of BoNT-A than those used on the magnetic beads are then added to the reaction. The binding of the multi-valent epitope on the magnetic beads by the antibodies on the B-cell surface leads to antibody clustering, which results in an intracellular calcium influx that activates the aequorin molecules and hence, luminescence. A luminometer detects the light output, which is expressed as relative light units (RLU) over time. Reported LODs for BoNT-A spiked into food and beverage samples ranged from 7.4 +/- 2.2 ng/mL (milk) to 171.9 +/- 64.7 ng/mL (viscous liquid egg).

Special Considerations: This method is listed as Tier I for presumptive analysis of BoNT-A in water samples and Tier II for aerosol, solid and particulate samples. The sample preparation procedures may facilitate analysis of other environmental sample types, although non-liquid samples will require aqueous extraction and clarification (e.g., centrifugation) prior to assay.

Source: Tam, C.C., Flannery, A.R. and Cheng, L.W. 2018. "Rapid, Sensitive, and Portable Biosensor Assay for the Detection of Botulinum Neurotoxin Serotype A in Complex Food Matrices." *Toxins*. 10(11): 476. https://doi.org/10.3390/toxins10110476

8.2.5.6 Confirmatory Analysis

Analytical Technique: LC-MS-MS (BoNT Serotypes A, B, E and F) and MALDI-TOF-MS (BoNT Serotypes A–G)

Method Developed for: BoNT Serotypes A, B, C, D, E, F and G in serum, stool and food
Method Selected for: These procedures have been selected for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to validate the procedures for environmental sample types.

Description of Method: This Endopep-MS assay has been developed to detect specific activities of all seven BoNT serotypes (A–G). Peptide products are cleaved by the enzymatic action of the BoNTs on four target peptide substrates in a reaction buffer created to maximize the enzymatic activity of the BoNT toxins. An inert peptide substrate is added as the internal standard, and the reaction mixture is incubated at 37°C for a minimum of 2 hours. If present, BoNT A–G will react with the target peptides to form cleaved peptide products that can be measured by LC-MS-MS with ESI or MALDI-TOF-MS, although the source cited below evaluated application of LC-MS-MS to measurement of only A, B, E and F. For MALDI-TOF-MS analysis, the reaction is quenched by the addition of a solution of α-cyano-4-hydroxycinnamic acid (CHCA) matrix. For LC-MS-MS analysis, the reaction is quenched by adding 5 µL of 10% acetic acid to the sample. A sample aliquot is then injected into and analyzed by LC-MS-MS, or spotted onto a MALDI sample plate and analyzed by MALDI-TOF-MS.

Quantification of the toxins is performed by comparing the area ratios of the unknowns to those of calibration standards. LOD concentrations are given in units of U/mL, where 1U is defined as the enzyme catalysis of 1 micromole (µmole) of substrate per minute, and mL represents the sample volume. LODs varied for all serotypes and ranged from 1.25 to 6.25 U/mL (MALDI-

TOF-MS) and 0.078 to 1.25 U/mL (LC-MS-MS) after four hours of incubation in the reaction buffer; and 0.313 to 6.25 U/mL (MALDI-TOF-MS) and 0.039 to 1.00 U/mL (LC-MS-MS) after incubation for 10 or 17 hours. The additional resource listed below (Kalb et al. 2015) describes a modified Endopep-MS assay, using antibody-based toxin capture followed by synthetic peptide cleavage and MALDI-TOF-MS detection. The MALDI-TOF-MS analysis required less than one minute to record the mass spectrum, and LODs in food matrices ranged from (depending on the serotype) 0.01 mouse lethal dose (mLD_{50}) to 0.75 mLD_{50}.

Special Considerations: This assay is listed as Tier II for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Only qualitative information (presence/absence) using MALDI-TOF-MS exists for BoNT-C, -D and -G, and LODs for these serotypes have not been reported using either LC-MS-MS or MALDI-TOF-MS. The sample preparation procedures used for food products also may be applicable to environmental sample types. Non-water samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Although procedures are not provided, the reference source notes that this method has also been used to test human stool and serum; and results indicate no false positives.

Source: Kalb, S.R., Krilich, J.C., Dykes, J.K., Luquez, C., Maslanka, S.E. and Barr, J.R. 2015. "Detection of Botulinum Toxins A, B, E, and F in Foods by Endopep-MS." *Journal of Agricultural and Food Chemistry*. 63(4): 1133-1141.
http://pubs.acs.org/doi/abs/10.1021/jf505482b

8.2.5.7 Analysis of Biological Activity

Analytical Technique: Mouse bioassay

Method Developed for: BoNTs in food products and clinical samples
Method Selected for: These procedures have been selected for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The mouse bioassay is the standard for government agencies testing for BoNT-containing food and clinical samples. Mice are injected with 0.5 mL sample, each sample dilution, or a heat-inactivated control sample. Mice are observed over a period of 1 to 7 days for symptoms of botulism and/or death. Results for the mouse bioassay are reported in median lethal dose (LD_{50}) units, where 1 LD_{50} is the amount of BoNT required to kill 50% of injected mice after a defined time interval. The mouse bioassay has a DL of 5 − 10 pg for BoNT serotype A. Some BoNTs are produced by non-proteolytic strains of *C. botulinum* (group II) and require trypsin activation prior to testing in the mouse bioassay. It is important to observe for symptoms of botulism in the test mice since death without clinical symptoms of botulism is not sufficient evidence that the material injected contained BoNT. BoNT typing can be conducted by injecting pairs of test mice with monovalent antitoxins prior to injecting with suspected toxin sample and noting a protective or neutralizing effect of the initial sample toxicity.

Special Considerations: This assay is listed as Tier I for analysis of the biological activity of BoNTs in aerosol, solid, particulate and water samples. It is a low-throughput, labor intensive assay and is not suitable for screening a large number of samples.

Source: Maslanka, S.E, Solomon, H.M., Sharma, S. and Johnson, E.A. 2015. "*Clostridium botulinum* and Its Toxins" *APHA Press Compendium of Methods for the Microbiological Examination of Foods*. Fifth Edition, Chapter 32: 397. Washington, DC: APHA Press.
https://secure.apha.org/imis/ItemDetail?iProductCode=978-087553-2738&CATEGORY=BK

8.2.6 Brevetoxins (BTX)

CAS RN: 98112-41-5 (A-type, congeners BTX-1, BTX-7, BTX-10), 79580-28-2 (B-type, congeners BTX-2, BTX-3, BTX-5, BTX-6, BTX-8, BTX-9)
Considered Variants: NA

Description: Suite of cyclic polyether neurotoxin compounds produced by a species of dinoflagellate, *Karenia brevis*.

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of Shellfish Research. 2020. 39(2): 491-500	Presumptive	Immunoassay (ELISA)	8.2.6.1
Toxicon. 2015. 96: 82-88	Confirmatory	LC-MS	8.2.6.2

8.2.6.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of brevetoxins (BTX) in shellfish
Method Selected for: This ELISA procedure has been selected for the presumptive analysis of BTX in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes single-laboratory validation of a commercially available ELISA for brevetoxins in shellfish extracts as well as comparison of ELISA results to the mouse bioassay. The ELISA is a competitive immunoassay format consisting of immobilized brevetoxin (BTX-3)-protein conjugate, polyclonal goat anti-brevetoxin antibodies, and HRP-linked secondary antibodies. Samples and goat anti-brevetoxin antibodies are combined with the BTX-3 -protein conjugate immobilized on microplate wells. Brevetoxins, when present in a sample, bind to the primary antibodies, making these antibodies unavailable for capture by the immobilized antigen (BTX-3). The addition of HRP-linked second antibody (anti-goat) and subsequent HRP-substrate (TMB) colorimetric reaction allows for quantitative assessment of anti-brevetoxin (goat) primary antibodies bound to the immobilized antigen. The intensity of the color is inversely proportional to the amount of brevetoxin that was present in the well during incubation. Results are expressed as mg BTX-3/g for spiked samples and mg BTX-3 equivalents/g for naturally incurred toxic samples. At a sample dilution of 1:400, the LOD and LOQ for brevetoxin in shellfish were 0.04 and 0.12 mg/g, respectively.

Special Considerations: This ELISA is listed as a Tier II procedure for presumptive analysis of brevetoxins in aerosol, solid, particulate and water samples. Sample preparation procedures used for shellfish suggest that similar extraction procedures may be applicable to environmental samples. It should be noted that this assay may underrepresent some brevetoxins in samples due to differential cross reactivities between the anti-brevetoxin antibodies and BTX congeners and metabolites. Only a few cross reactivities have been reported. The ELISA is based on antibodies thought to have higher specificity for B-type BTX (identified in Appendix D). Results are reported in units of BTX-3 equivalents. BTX-3 is formed from its parent BTX-2, which is reported to dominate naturally occurring BTX incidents. BTX-2 and BTX-9 are reported to have similar cross-reactivity as BTX-3, but BTX-1 can exhibit cross reactivity as low as a few percent. Metabolites, even of B-type BTX, can similarly vary from a few percent to similar cross reactivity, as well. ***Note***: The role of this ELISA should consider the specific BTX/metabolites of interest during environmental remediation.

Source: Flewelling, L.J, Corcoran, A.A., Granholm, A.A., Takeuchi, N Y., Van Hoeck, R.V., Zahara, M.L. 2020. "Validation and Assessment of an Enzyme-Linked Immunosorbent Assay (Elisa) for Use in Monitoring and Managing Neurotoxic Shellfish Poisoning." *Journal of Shellfish Research*. 39(2): 491-500. https://doi.org/10.2983/035.039.0230

8.2.6.2 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: Determination of brevetoxins in shellfish
Method Selected for: These procedures have been selected for confirmatory analysis of brevetoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involved extraction of clam tissue homogenates with either acetone or 80% methanol in water. Extracts are then defatted using 95% n-hexane, followed by SPE using a C_{18} column. The SPE eluent is evaporated to dryness under nitrogen, redissolved in methanol at a concentration of 0.5 g tissue/mL of solution, filtered through a syringe filter and analyzed by LC-MS equipped with a C_8 column.

Special Considerations: This method is listed as a Tier II procedure for confirmatory analysis of brevetoxin in aerosol, solid, particulate and water samples. The procedures described in the source method are for extraction of clam homogenates (Abraham, 2015). Additional resource citation listed below (Abraham, 2006) includes extraction procedures for aerosol filters, shellfish, laboratory cultures and natural blooms of *Karenia brevis,* which may be applicable to water, soil, aerosol filter and particulate sample types.

Source: Abraham, A., El Said, K.R., Wang, Y., Jester, E.L.E., Plakas, S.M., Flewelling, L.J., Henry, M.S. and Pierce, R.H. 2015. "Biomarkers of brevetoxin exposure and composite toxin levels in hard clam (Mercenaira sp.) exposed to Karenia brevis blooms." *Toxicon*. 96: 82-88. https://www.ncbi.nlm.nih.gov/pubmed/25620222

Additional Resource: Abraham, A., Plakas, S.M., Wang, Z., Jester, E.L.E., El Said, K.R., Granade, H.R., Henry, M.S., Blum, P.C., Pierce, R.H. and Dickey, R.W. 2006. "Characterization of polar brevetoxin derivatives isolated from *Karenia brevis* cultures and natural blooms." *Toxicon*. 48: 104-115.
http://www.sciencedirect.com/science/article/pii/S0041010106001632

8.2.7 α-Conotoxins

CAS RN: Various
Considered Variants: NA

Description: Small disulfide rich peptides present in the venom of predatory marine snails of the genus *Conus*.

Selected Methods	Analysis Type*	Analytical Technique	Section
Toxins. 2017. 9(9): 281	Confirmatory	LC-MS	8.2.7.1

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.7.1 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: Monitoring the physical properties of α-conotoxins in response to various reagents used for decontamination
Method Selected for: This procedure has been selected for confirmatory analysis of α-conotoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: α-Conotoxins are analyzed by HPLC-MS. The electrospray ionization source is operated in positive ion mode. Samples are analyzed by direct infusion using a syringe pump or by HPLC separation on a C18 column using a linear gradient of acetonitrile (5%−65%) in 1% formic acid in water.

Special Considerations: This procedure is listed as Tier III for confirmatory analysis of α-conotoxins in aerosol, solid, particulate and water samples. Sample preparation procedures have not been evaluated for environmental samples. The specific MS or MS-MS conditions, including *m/z* and MS-MS transitions monitored, should be based on the specific α-conotoxin(s) of interest during environmental remediation.

Source: Turner, M.W., Cort, J.R. and McDougal, O.M. 2017. "α-Conotoxin Decontamination Protocol Evaluation: What Works and What Doesn't." *Toxins*. 9(9): 281. https://doi.org/10.3390/toxins9090281

8.2.8 Cylindrospermopsin

CAS RN: 143545-90-8
Considered Variants: NA

Description: Polycyclic uracil derivative containing guanidino and sulfate groups produced by a variety of freshwater cyanobacteria.

Selected Methods	Analysis Type	Analytical Technique	Section
Environ. Sci. Technol. 44: 7361-7368	Presumptive	Immunoassay (ELISA)	8.2.8.1
EPA Method 545	Confirmatory	LC-MS-MS	8.2.8.2
EPA/600/R-17/130	Confirmatory	LC-MS-MS	8.2.8.3

8.2.8.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Cylindrospermopsin in ground water, surface water and well water
Method Selected for: These procedures have been selected for presumptive analysis of cylindrospermopsin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Cylindrospermopsin is detected using a commercially available colorimetric immunoassay (competitive ELISA) procedure. Cyanobacterial cells in the sample are lysed by three sequential freeze-thaw cycles to allow determination of total toxin. Sample (0.05 mL), enzyme conjugate (cylindrospermopsin- HRP), and an antibody solution containing rabbit anti-cylindrospermopsin antibodies are added to plate wells containing immobilized sheep anti-rabbit antibodies. Both the cylindrospermopsin (if present) in the sample and cylindrospermopsin-HRP conjugate compete in solution to bind to the rabbit anti-cylindrospermopsin antibodies in proportion to their respective concentrations. After incubation, the unbound molecules are washed and decanted. A specific substrate is then added which is converted from a colorless to a blue solution by the HRP enzyme conjugate solution. The reaction is terminated with the addition of a dilute acid. The concentration of cylindrospermopsin in the sample is determined photometrically by comparing sample absorbance to the absorbance of calibrators at a specific wavelength (450 nm). The applicable concentration range is 0.4–2.0 µg/L, with a minimum detection level of 0.4 µg/L.

Special Considerations: This method is listed as Tier II for presumptive analysis of cylindrospermopsin in aerosol, solid, particulate and water samples. The source citation listed

below (Graham, 2010) details the use of this ELISA for assessing cylindrospermopsin in naturally occurring freshwater cyanobacterial blooms. Non-aqueous samples will require an aqueous extraction procedure prior to assay. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Graham, J.L., Loftin, K.A., Meyer, M.T. and Ziegler, A.C. 2010. "Cyanotoxin Mixtures and Taste-and-Odor Compounds in Cyanobacterial Blooms from the Midwestern United States." *Environmental Science and Technology*. 44: 7361-7368. https://pubs.acs.org/doi/10.1021/es1008938 (***Note***: Descriptive lake data, analytical details for LC/MS/MS, cyanobacterial community composition data, and dissolved toxin data are available at: http://pubs.acs.org/.)

8.2.8.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Cylindrospermopsin in finished drinking water
Method Selected for: This method has been selected for confirmatory analysis of cylindrospermopsin in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method is used for detection of cylindrospermopsin in drinking water samples. Samples are frozen and thawed three times, then filtered. A sample aliquot from the filtrate is combined with internal standards and analyzed using LC-MS-MS with ESI. This method requires the use of MS-MS in MRM mode to enhance selectivity. Cylindrospermopsin is identified by comparing retention times and signals produced by unique mass transitions to those of procedural calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports an LCMRL of 0.063 µg/L for cylindrospermopsin in fortified reagent water. The working range reported in the method is 0.050–10.0 µg/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of cylindrospermopsin in drinking water and Tier II for confirmatory analysis of cylindrospermopsin in aerosol, solid and particulate samples. Non-aqueous samples will require additional extraction procedures. SPE also may be needed for additional cleanup and concentration of these sample types. Extraction of cylindrospermopsin from ground-up cyanobacterial mat material has been reported (Wood, 2008). An additional resource below (Haddad et al. 2019) describes liquid-liquid extraction and SPE cleanup procedures for fish tissue that may facilitate preparation of non-aqueous environmental samples. These procedures might be modified for analysis of soil, aerosol and particulate samples using the LC-MS-MS conditions described in this method.

Source: U.S. EPA. April 2015. Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). Washington, DC: U.S. EPA. EPA 815-R-15-009. https://www.epa.gov/sites/default/files/2017-10/documents/epa_815-r-15-009_method_545.pdf

Additional Resources:

- Wood, S., Mountfourt, D., Selwood, A., Holland, P., Puddick, J. and Cary, S. 2008. Widespread Distribution and Identification of Eight Novel Microcystins in Antarctic Cyanobacterial Mats. *Applied Environmental Microbiology*. 74(23): 7243-7251. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2592942/
- Haddad S.P., Bobbitt J.M., Taylor R.B., Lovin, L.M., Conkle, J.L., Chambliss, C.K. and Brooks, B.W. 2019. "Determination of microcystins, nodularin, anatoxin-a, cylindrospermopsin, and saxitoxin in water and fish tissue using isotope dilution liquid chromatography tandem mass spectrometry." *Journal of Chromatography A*. 1599:66-74.

https://doi.org/10.1016/j.chroma.2019.03.066

8.2.8.3 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Cylindrospermopsin and anatoxin-a in ambient freshwaters
Method Selected for: This method has been selected for confirmatory analysis of cylindrospermopsin in non-drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than untreated freshwater.

Description of Method: This method uses LC-MS-MS for determination of cylindrospermopsin and anatoxin-a (combined intracellular and extracellular) in ambient freshwater. Samples are subjected to three freeze/thaw cycles, internal standard is added, and the sample is filtered. Samples with significant cell densities may require centrifugation prior to filtration. An aliquot of the sample filtrate is injected into an LC equipped with an analytical column that is interfaced to an MS-MS capable of positive ion electrospray ionization (ESI). The analytes are separated and identified by comparing retention times and signals produced by unique mass transitions to retention times and mass transitions for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports a LCMRL of 0.23 µg/L and calculated DL of 0.065 µg/L for cylindrospermopsin in fortified reagent water.

Special Considerations: This method is listed as Tier I for confirmatory analysis of cylindrospermopsin in non-drinking water samples.

Source: U.S. EPA. November 2017. "Determination of Cylindrospermopsin and Anatoxin-a in Ambient Freshwaters by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-17/130.
https://www.epa.gov/water-research/single-laboratory-validated-method-determination-cylindrospermopsin-and-anatoxin

8.2.9 Deoxynivalenol

CAS RN: 51481-10-8
Considered Variants: NA
Description: Trichothecene mycotoxin produced by *Fusarium* spp.

Selected Methods	Analysis Type*	Analytical Technique	Section
Journal of Agricultural and Food Chemistry. 2017. 65(33): 7138-7152.	Confirmatory	LC-MS-MS	8.2.9.1

*At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.9.1 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins (including aflatoxins, deoxynivalenol, fumonisin, ochratoxin A and zearalenone) in corn, peanut butter and wheat flour
Method Selected for: These procedures have been selected for confirmatory analyses of deoxynivalenol in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes a collaborative laboratory study to evaluate an LC-MS-MS procedure using commercially available ^{13}C-labeled internal standards for simultaneous detection and quantification of multiple mycotoxins. The method described can be used to detect and quantify mycotoxins including: aflatoxins; deoxynivalenol; fumonisins B1, B2, and B3; ochratoxin A; and zearalenone. Procedures for sample fortification, extraction, filtration and centrifugation are described in addition to LC-MS-MS conditions and parameters for various platforms used by laboratories participating in the study. The ranges of analytical performance for the six laboratories depended on LC-MS instrument conditions (column injection volume, flow rate, etc.). Average recoveries of the participating laboratories were in the range of 90−110%, with repeatability RSD_r (within laboratory) < 10% and reproducibility RSD_R (among laboratories) < 15%. LOQ range for deoxynivalenol was 0.1−5.0 ng/mL.

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of deoxynivalenol in aerosol, solid, particulate and water samples. The sample preparation procedures described for food/feed (extraction with acetonitrile/water, centrifugation, and filtration) may be applicable to environmental samples.

Source: Zhang, K., Schaab, M.R., Southwood, G., Tor, E.R., Aston, L.S., Song, W., Eitzer, B., Majumdar, S., Lapainis, T., Mai, H., Tran, K., El-Demerdash, A., Vega, V., Cai, Y., Wong, J.W., Krynitsky, A.J. and Begley, T.H. 2017. "Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)." *Journal of Agricultural and Food Chemistry*. 65(33): 7138-7152. https://doi.org/10.1021/acs.jafc.6b04872

8.2.10 Domoic Acid (DA)

CAS RN: 14277-97-5
Considered Variants: NA

Description: Water-soluble, nonprotein amino acid neurotoxin produced by microscopic algae, specifically the diatom species *Pseudo-nitzschia.* Composed of a proline ring, one imino group and three carboxyl groups.

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of AOAC International. 2007. 90(4): 1011-1027	Presumptive	Immunoassay (ELISA)	8.2.10.1
Journal of Shellfish Research. 2008. 27(5): 1301-1310	Presumptive	Immunoassay (ELISA)	8.2.10.2
See Section 8.2.10.3	Presumptive	Immunoassay (LFA)	8.2.10.3
Journal of AOAC International. 2014. 97(2): 316-324	Confirmatory	LC-MS	8.2.10.4

8.2.10.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of DA in shellfish
Method Selected for: These procedures have been selected for presumptive analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available ELISA assay is used to determine DA in shellfish. The ELISA assay is a direct competitive format, where free DA in the sample competes with DA-conjugated protein coated on plastic wells for binding to anti-DA antibodies free in the solution. Resulting color intensity is measured spectrophotometrically on a plate reader at 450 nm, and is inversely proportional to the concentration of DA in the sample solution. The assay is

calibrated using dilutions of a DA standard. The LOD for shellfish extracts is 0.01 mg/kg. Based on the results of a collaborative study (Kleivdal et al. 2007), the ELISA kit is under consideration as an official AOAC method.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of DA in aerosol, solid, particulate and water samples. The methanol/water extraction method used for shellfish may be applicable to non-aqueous environmental sample types. Liquid samples may only require dilution prior to assay while those containing intact organisms (e.g., phytoplankton), may require cell disruption prior to dilution and assay. Results of a multi-laboratory study are described in the source reference (Kleivdal et al. 2007) and indicate that the ELISA method may slightly overestimate DA levels in shellfish when compared to LC-MS (see additional resource citation, Quilliam et al. 1995).

Source: Kleivdal, H., Kristiansen, S.I., Nilsen, M.V., Goksoyr, A., Briggs, L., Holland, P. and McNabb, P. 2007. "Determination of Domoic Acid Toxins in Shellfish by Biosense ASP ELISA – A Direct Competitive Enzyme-Linked Immunosorbent Assay: Collaborative Study." J*ournal of AOAC International*. 90(4): 1011-1027. https://doi.org/10.1093/jaoac/90.4.1011

Additional Resource: Quilliam, M.A., Xie, M. and Hardstaff, W.R. 1995. "Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood." *Journal of AOAC International*. 78(2): 543-554. https://doi.org/10.1093/jaoac/78.2.543

8.2.10.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: DA in shellfish
Method Selected for: These procedures have been selected for presumptive analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available ELISA test kit is used for detecting DA using a mAbs. The sequential competitive ELISA gives equivalent results to those obtained using standard HPLC, fluorenylmethoxycarbonyl HPLC, or liquid chromatography-mass spectrometry (LC-MS) methods. It has a linear range from 0.1 to 3 ppb and was used to measure DA in razor clams, mussels, scallops, and phytoplankton. The assay requires approximately 1.5 h to complete and has a standard 96-well format.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of DA in aerosol, solid, particulate and water samples. The methanol/water extraction method used for shellfish may be applicable to non-aqueous environmental sample types. Liquid samples may only require dilution prior to assay while those containing intact organisms (e.g., phytoplankton), may require cell disruption prior to dilution and assay.

Source: Litaker, R.W., Stewart, T. N., Eberhart, B-T. L., Wekell, J.C., Trainer, V.L., Kudela, R.M., Miller, P.E., Roberts, A., Hertx, C., Johnson, T.A., Frankfurter, G., Smith, G.J., Schnetzer, A., Schumacker, J., Bastian, J.L., Odell, A., Gentien, P., Le Gal, D., Hardison, D.R. and Tester, P.A. 2008. "Rapid Enzyme-Linked Immunosorbent Assay for Detection of the Algal Toxin Domoic Acid." *Journal of Shellfish Research*. 27(5): 1301-1310.
http://www.bioone.org/doi/abs/10.2983/0730-8000-27.5.1301

8.2.10.3 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: Determination of DA in shellfish
Method Selected for: These procedures have been selected for presumptive analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available LFA is used to determine DA in shellfish. The assay is a single-step lateral flow device based on a competitive immunoassay format. Following a simple distilled water extraction of homogenized shellfish tissue, the extract is diluted in running buffer, and the dipstick-format device is placed into the diluted extract. The extract is wicked through a reagent zone containing antibodies specific for DA conjugated to colloidal gold particles. If DA is present, it will be captured by the labeled antibody. Migration of the sample continues through a membrane, which contains a zone of DA conjugated to a protein carrier. This zone captures any unbound antibody-gold conjugate, resulting in a visible line. With increasing amounts of DA in the test sample, less unbound conjugate is available for binding to the test line. Thus, intensity of the test line is inversely proportional to the amount of DA in the sample. The test device also incorporates a control conjugate, which binds to a second line. The control line will form regardless of the amount of DA present in the sample, ensuring that the test device is functioning properly. Results are analyzed as positive or negative using a commercial strip reader. The LFA is intended for the qualitative screening of shellfish for DA, by producing a positive result with samples containing 20 ppm or above.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of DA in aerosol, solid, particulate and water samples. The water extraction method used for shellfish may be applicable to non-aqueous environmental sample types. Liquid samples may only require dilution prior to assay.

Source: Caballero, O., Melville, K., Gray, L., Jawaid, W., Hooper, M., Muirhead, P., Mozola, M. and Rice, J. 2013. "Validation Study of the Reveal® 2.0 ASP Test for the Qualitative Detection of Domoic Acid in Shellfish."
https://www.issc.org/Data/Sites/1/media/labreferencepage/reveal-2.0-asp__e33__13-112-summary-of-actions-with-slv.pdf

8.2.10.4 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: DA in shellfish
Method Selected for: These procedures have been selected for confirmatory analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This LC-MS method for the analysis of DA and lipophilic toxins in shellfish homogenates was developed using a hybrid triple quadrupole linear ion trap MS. Prior to extraction, portions (2.0 ± 0.2 g) of homogenized samples are weighed in 50-mL centrifuge tubes and mixed for 3 min with 9 mL of methanol using a vortex mixer. The supernatant is removed following centrifugation. An additional 9 mL of methanol is added to the remaining sample pellet and extracted for 1 minute using a Polytron. After centrifugation, the supernatant is combined with that from the first step in a 20-mL flask and brought to volume with methanol. Aliquots of final extracts are filtered (0.45 µm) before further work. A 1-mL portion of extract is placed in a 1.5-mL HPLC vial, to which 125 µL of 2.5 M NaOH is then added and the solution vortex mixed. The vials are capped tightly and heated for 40 minutes at 76°C. When cool, the samples are neutralized with 125 µL of 2.5 M HCl and vortex mixed. Hydrolyzed samples are filtered (0.45 µm) prior to analysis. For routine quantitation, a scheduled selected reaction monitoring method

is used for the analysis of DA. The estimated LOD reported for DA in shellfish homogenates is 10 µg/kg.

Special Considerations: This method is listed as Tier II for confirmatory analysis of DA in aerosol, solid, particulate and water samples, owing to similarities between typical sample processing steps for these types of samples and the sample processing reported in this method. However, the procedures described in this method may need to be modified for water, soil, aerosol and particulate samples.

Source: McCarron, P., Wright, E., and Quilliam, M.A. 2014. "Liquid Chromatography/Mass Spectrometry of Domoic Acid and Lipophilic Shellfish Toxins with Selected Reaction Monitoring and Optional Confirmation by Library Searching of Product Ion Spectra." *Journal of AOAC International*. 97(2): 316-324. https://doi.org/10.5740/jaoacint.SGEMcCarron

8.2.11 Fumonisin

CAS RNs: 116355-83-0 (B1), 116355-84-1 (B2), 136379-59-4 (B3)
Considered Variants: B1, B2, B3

Description: Mycotoxins produced by several Fusarium fungi. Fumonisins are polyhydroxyl alkylamines esterified with two carbon acids and differ by the presence and position of the free hydroxyl groups.

Selected Methods	Analysis Type*	Analytical Technique	Section
Journal of Agricultural and Food Chemistry. 2017. 65(33): 7138-7152	Confirmatory	LC-MS-MS	8.2.11.1

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.11.1 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins (including aflatoxins, deoxynivalenol, fumonisin, ochratoxin A and zearalenone) in corn, peanut butter, and wheat flour
Method Selected for: These procedures have been selected for confirmatory analyses of fumonisin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes a collaborative laboratory study to evaluate an LC-MS-MS procedure using commercially available ^{13}C-labeled internal standards for simultaneous detection and quantification of multiple mycotoxins. The method described can be used to detect and quantify mycotoxins including: aflatoxins; deoxynivalenol; fumonisins B1, B2, and B3; ochratoxin A; and zearalenone. Procedures for sample fortification, extraction, filtration and centrifugation are described in addition to LC-MS-MS conditions and parameters for various platforms used by laboratories participating in the study. The ranges of analytical performance for the six laboratories depended on LC-MS instrument conditions (column injection volume, flow rate, etc.). The average recoveries of the participating laboratories were in the range of 90−110%, with repeatability RSD_r (within laboratory) < 10% and reproducibility RSD_R (among laboratories) < 15%. The LOQs ranges were: fumonisin B1 (0.1−2.5 ng/mL), fumonisin B2 (0.05−5.0 ng/mL), fumonisin B3 (0.1−5.0 ng/mL).

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of fumonisin in aerosol, solid, particulate and water samples. The sample preparation procedures

described for food/feed (extraction with acetonitrile/water, centrifugation, and filtration) may be applicable to environmental samples.

Source: Zhang, K., Schaab, M.R., Southwood, G., Tor, E.R., Aston, L.S., Song, W., Eitzer, B., Majumdar, S., Lapainis, T., Mai, H., Tran, K., El-Demerdash, A., Vega, V., Cai, Y., Wong, J.W., Krynitsky, A.J. and Begley, T.H. 2017. "Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)." *Journal of Agricultural and Food Chemistry*. 65(33): 7138-7152. https://doi.org/10.1021/acs.jafc.6b04872

8.2.12 Microcystins

CAS RNs: 96180-79-9 (LA), 154037-70-4 (LF), 101043-37-2 (LR), 123304-10-9 (LY), 111755-37-4 (RR), 101064-48-6 (YR)
Considered Variants: LA, LF, LR, LY, RR, YR

Description: Cyclic heptapeptide hepatotoxins produced by a variety of freshwater cyanobacteria.

Selected Methods	Analysis Type	Analytical Technique	Section
EPA Method 546	Presumptive	Immunoassay (ELISA)	8.2.12.1
EPA Method 544	Confirmatory	LC-MS-MS	8.2.12.2
EPA/600/R-17/344	Confirmatory	LC-MS-MS	8.2.12.3
Toxins. 2019. 11(12): 729	Biological Activity	Protein Phosphatase 2A (PP2A) Activity Assay	8.2.12.4

8.2.12.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Microcystins (MC) in water
Method Selected for: This method has been selected for presumptive analysis of microcystins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: EPA Method 546 determines total microcystins (MC) and nodularins (NOD) in finished drinking water and in ambient water using a commercially available indirect competitive ELISA. The term "total microcystins and nodularins" is defined as the sum of the congener-independent, intracellular and extracellular microcystins and nodularins that are measurable in a sample. Cyanobacterial cells in the sample are lysed by three sequential freeze-thaw cycles to allow determination of total toxin, and the total concentration of toxin is measured based on detection of a characteristic feature common to microcystin and nodularin congeners (structural variants), specifically, the Adda amino acid side chain (see Section 1.1 of Method 546). To ensure comparability between laboratories, the ELISA is calibrated against one congener, MC-LR. The minimum reporting level (MRL) for MC-LR is 0.30 µg/L. The method describes required laboratory QC and demonstration of capability procedures.

Special Considerations: This method is listed as Tier I for presumptive analysis of microcystins in water samples and Tier II for presumptive analysis of microcystins in all other environmental sample types. Non-aqueous samples will require aqueous extraction prior to analysis. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular MC.

Source: Zaffiro, A., Rosenblum, L. and Wendelken, S. 2016. "Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-

Linked Immunosorbent Assay." Washington, DC: U.S. EPA. https://www.epa.gov/sites/default/files/2016-09/documents/method-546-determination-total-microcystins-nodularins-drinking-water-ambient-water-adda-enzyme-linked-immunosorbent-assay.pdf

8.2.12.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of microcystins and nodularin in drinking water
Method Selected for: This method has been selected for confirmatory analysis of microcystins in drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: EPA Method 544 determines six microcystins (including MC-LR) and nodularin in drinking water using SPE and LC-MS-MS. Samples are fortified with a surrogate, filtered, and the filter is placed in a methanol solution to release toxin. The sample filtrate and methanol solution are combined, passed through an SPE cartridge, eluted, evaporated to dryness, and redissolved in methanol solution. A 10-µL aliquot of extracted sample is analyzed by LC-MS-MS equipped with a C_8 column. Microcystins are identified by comparing the acquired mass spectra and retention times to those of calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by external standard calibration. DLs for analytes in this method range from 1.2 to 4.6 ng/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of microcystins in drinking water. It may be possible to analyze relatively clean water samples for extracellular toxins by direct injection into an LC-MS-MS; however, dirty water samples or water samples with low concentrations of toxin may require cleanup and concentration using SPE.

Source: Shoemaker, J., Tettenhorst, D. and Delacruz, A. 2015. "Method 544: Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)," Version 1.0. Cincinnati, OH: EPA. EPA/600/R-14/474. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=306953

8.2.12.3 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of microcystins and nodularin in ambient fresh water
Method Selected for: This method has been selected for confirmatory analysis of microcystins in aerosol, solid, particulate and non-drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than fresh water.

Description of Method: This method describes an LC-MS-MS procedure for determination of microcystins and nodularin (combined intracellular and extracellular) in ambient freshwater. A water sample is filtered and intracellular toxins are released from cyanobacterial cells following two possible procedures chosen by visual transparency or cell density of the sample. The filtered sample, containing intracellular and extracellular toxins, is passed through an SPE cartridge to extract the target analytes and surrogate. Analytes are eluted from the solid phase with 90:10 methanol:reagent water (v/v). The extract is concentrated to dryness by evaporation with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 90:10 methanol:reagent water (v/v). 10-µL is injected into an LC equipped with a C8 column that is interfaced to an MS-MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by internal standard calibration. DLs for analytes in this method range from 2.1 to 33 ng/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of microcystins in non-drinking water and Tier II for presumptive analysis of microcystins in all other environmental sample types. Non-aqueous samples will require aqueous extraction prior to analysis. Samples containing intact cyanobacteria must be treated to disrupt the cells to recover intracellular MCs. The additional resource listed below (Haddad et al. 2019*)* describes liquid-liquid extraction and SPE cleanup procedures for fish tissue that may facilitate preparation of non-aqueous environmental samples. This resource also details procedures for LC-MS-MS detection and quantitation (isotope dilution) of MCs. In addition, the sample preparation procedures described by Parker et al. (see additional resource below) for algal dietary supplements may be applicable to environmental samples.

Source: Shoemaker, J.A., Tettenhorst, D.R. and de la Cruz, A. 2017. "Single Laboratory Validated Method for Determination of Microcystins and Nodularin in Ambient Freshwaters by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." Cincinnati, OH: EPA. EPA/600/R-17/344. https://www.epa.gov/water-research/single-laboratory-validated-method-determination-microcystins-and-nodularin-ambient

Additional Resources:

- Haddad S.P., Bobbitt J.M., Taylor R.B., Lovin, L.M., Conkle, J.L., Chambliss, C.K. and Brooks, B.W. 2019. "Determination of microcystins, nodularin, anatoxin-a, cylindrospermopsin, and saxitoxin in water and fish tissue using isotope dilution liquid chromatography tandem mass spectrometry." *Journal of Chromatography A*. 1599: 66-74. https://doi.org/10.1016/j.chroma.2019.03.066
- Parker, C.H., Stutts, W.L. and DeGrasse, S.L. 2015. "Development and Validation of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Quantitation of Microcystins in Blue-Green Algal Dietary Supplements." *Journal of Agricultural and Food Chemistry*. 63 (47): 10303-10312. https://pubs.acs.org/doi/10.1021/acs.jafc.5b04292

8.2.12.4 Analysis of Biological Activity

Analytical Technique: Protein Phosphatase 2A (PP2A) Activity Assay

Method Developed for: Microcystins and nodularin PP2A activity in urine
Method Selected for: These procedures have been selected for biological activity analysis of microcystins (MCs) in aerosol, solid, particulate, and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference (cited below) describes the development and subsequent validation of an immunocapture-protein phosphatase inhibition assay to detect and measure combined inhibitory activity of MCs and nodularin. Immunocapture and concentration of MCs is accomplished using adda-specific antibodies coupled to magnetic beads. Antibody-bound MCs are eluted from the bead complex and subjected to a PP2A inhibition assay. Inhibition of PP2A activity is quantified by absorbance measurement (A450) of colorimetric substrate utilization, which decreases in the presence of MCs. The reported method quantitation range for MC-LR was 0.050–0.500 ng/mL, and the calculated method LOD was 0.0283 ng/mL. Other MC congeners (and nodularin) can also be measured in equivalents relative to MC-LR. Reagents for both immunocapture and PP2A assay, along with certified standards (e.g., MC-LR), are commercially available.

Special Considerations: This method is listed as Tier II for biological activity analysis of MCs in aerosol, solid, particulate and water samples. Immunocapture and concentration of MCs may be applicable to other environmental samples or sample extracts. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to immunocapture.

Source: Wharton, R.E., Cunningham, B.R., Schaefer, A.M., Guldberg, S.M., Hamelin, E.I. and Johnson, R.C. 2019. "Measurement of Microcystin and Nodularin Activity in Human Urine by Immunocapture-Protein Phosphatase 2A Assay." *Toxins*. 11(12): 729. https://dx.doi.org/10.3390%2Ftoxins11120729

8.2.13 Ochratoxin A

CAS RNs: 303-47-9
Considered Variants: NA

Description: Ochratoxins are derivatives of an isocoumarin moiety linked to phenylalanine by an amide bond produced by *Penicillium verrucosum* and different species of *Aspergillus molds.*

Selected Methods	Analysis Type*	Analytical Technique	Section
Journal of Agricultural and Food Chemistry. 2017. 65(33): 7138-7152	Confirmatory	LC-MS-MS	8.2.13.1

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.13.1 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins (including aflatoxins, deoxynivalenol, fumonisin, ochratoxin A and zearalenone) in corn, peanut butter, and wheat flour
Method Selected for: These procedures have been selected for confirmatory analyses of ochratoxin A in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes a collaborative laboratory study to evaluate an LC-MS-MS procedure using commercially available ^{13}C-labeled internal standards for simultaneous detection and quantification of multiple mycotoxins. The method described can be used to detect and quantify mycotoxins including: aflatoxins; deoxynivalenol; fumonisins B1, B2, and B3; ochratoxin A; and zearalenone. Procedures for sample fortification, extraction, filtration and centrifugation are described in addition to LC-MS-MS conditions and parameters for various platforms used by laboratories participating in the study. The ranges of analytical performance for the six laboratories depended on LC-MS instrument conditions (column injection volume, flow rate, etc.). For example, average recoveries of the participating laboratories were in the range of 90−110%, with repeatability RSD_r (within laboratory) < 10% and reproducibility RSD_R (among laboratories) < 15%. LOQ range for ochratoxin A was 0.02−2.5 ng/mL.

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of ochratoxin A in aerosol, solid, particulate and water samples. The sample preparation procedures described for food/feed (extraction with acetonitrile/water, centrifugation, and filtration) may be applicable to environmental samples.

Source: Zhang, K., Schaab, M.R., Southwood, G., Tor, E.R., Aston, L.S., Song, W., Eitzer, B., Majumdar, S., Lapainis, T., Mai, H., Tran, K., El-Demerdash, A., Vega, V., Cai, Y., Wong, J.W., Krynitsky, A.J. and Begley, T.H. 2017. "Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)." *Journal of Agricultural and Food Chemistry*. 65(33): 7138-7152. https://doi.org/10.1021/acs.jafc.6b04872

8.2.14 Picrotoxin

CAS RN (Picrotoxin): 124-87-8
CAS RN (Picrotin): 21416-53-5
CAS RN (Picrotoxinin): 17617-45-7
Considered Variants: NA

Description: Alkaloid toxin produced by the climbing plant, *Anamirta cocculus*, and consisting of picrotin and picrotoxinin.

Selected Methods	Analysis Type*	Analytical Technique	Section
Journal of Pharmaceutical and Biomedical Analysis. 1989. 7(3): 369-375	Confirmatory	LC-UV	8.2.14.1

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.14.1 Confirmatory Analysis

Analytical Technique: LC-UV

Method Developed for: Picrotoxin in serum
Method Selected for: These procedures have been selected for confirmatory analysis of picrotoxin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Picrotoxin (picrotin and picrotoxinin) is quantified in serum by reversed phase HPLC. Serum samples are prepared by washing with *n*-hexane, followed by extraction with chloroform. The chloroform is evaporated and the sample is reconstituted in acetonitrile-1 mM ammonium acetate buffer (pH 6.4) 34:66 (v/v) for assay. The effluent is monitored at 200 nm, and quantification is based on peak-height ratio of analyte to the internal standard. A linear response is obtained for both analytes (picrotin and picrotoxinin) in the range of 0.2 to 20.0 µg/mL with mean recoveries > 94.2%. Increased sensitivity may be possible using LC-TOF-MS analyses (Ogawa et al. 2016).

Special Considerations: This method is listed as Tier II for confirmatory analysis of picrotoxin in aerosol, solid, particulate and water samples. The procedures described may need to be modified for water, soil, aerosol and particulate samples, such as use of agitation (e.g., shaking, ultrasonication). The extraction solvent specified (chloroform) may be suitable for these sample types, but has not been evaluated. Extracts also may require cleanup and concentration by SPE. Although it may be possible to analyze relatively clean water samples by direct injection into a reversed-phase LC, dirty water samples or water samples with low levels of picrotoxin may require cleanup and concentration using SPE.

Source: Soto-Otero, R., Mendez-Alvarez, E., Sierra-Paredes, G., Galan-Valiente, J., Aguilar-Veiga, E. and Sierra-Marcuno, G. 1989. "Simultaneous Determination of the Two Components of Picrotoxin in Serum by Reversed-Phase High-Performance Liquid Chromatography With Application to a Pharmacokinetic Study in Rats." *Journal of Pharmaceutical & Biomedical Analysis*. 7(3): 369-375.
http://www.sciencedirect.com/science/article/pii/0731708589801049

Additional Resource: Ogawa, T., Tada, M., Hattori, H., Shiraishi, Y., Suzuki, T., Iwai, M., Kusano, M., Zaitsu, K., Ishii, A. and Seno, H. May 2016. "Sensitive determination of picrotoxin by liquid chromatography-quadrupole time-of-flight mass spectrometry." Letter to the Editor. *Legal Medicine*. 20: 8-11. https://doi.org/10.1016/j.legalmed.2016.03.002

8.2.15 Ricin (Ricinine)

Ricin – CAS RN: 9009-86-3
Description: Toxic lectin (carbohydrate-binding protein) found in the seeds of the castor oil plant, *Ricinus communis*. 60 kDa glycoprotein consisting of a deadenylase (~32 kDa A chain) and lectin (~34 kDa B chain); an agglutinin of MW 120 kDa may be present in crude castor bean preparations.

Ricinine – CAS RN: 5254-40-3
Description: Small molecule, alkaloid marker for ricin.

Selected Methods	Analysis Type	Analytical Technique	Section
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 2013. 11 (4): 237−250	Presumptive	Immunoassay (LFA)	8.2.15.1
Journal of Food Protection. 2005. 68(6): 1294–1301	Presumptive	Immunoassay (ELISA)	8.2.15.2
EPA/600/R-22/033A	Presumptive	Immunoassay (ECL)	8.2.15.3
EPA 600/R-13/022 (EPA/CDC)	Presumptive	LC-MS-MS	8.2.15.4
CDC LRN*	Presumptive	Time-Resolved Fluorescence (TRF) Immunoassay	—
Analytical Chemistry. 2011. 83: 2897−2905	Confirmatory	Immunocapture / LC-MS-MS	8.2.15.5
Analytical Chemistry. 2016. 88: 6867-6872	Biological Activity	Immunocapture / MALDI-TOF-MS	8.2.15.6

*A standardized procedure, reagents and agent-specific algorithms are available only to LRN member laboratories (see Section 7.1.4 for more information on the LRN).

8.2.15.1 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: Ricin in buffer, food products, powders and aerosol filter extracts
Method Selected for: These procedures have been selected for presumptive analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This lateral flow immunochromatographic device uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second line in the control window indicates that the test strip functioned properly. Two colored lines (in the sample and control windows) are required for a positive result determination. To perform a test on a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and can be read visually or with a reader.

The source reference (below) details a multicenter evaluation of the sensitivity, specificity, reproducibility, and limitations of an LFA for ricin that can be used in the field or in the laboratory to qualitatively screen for ricin in environmental samples. Using the recommended test strip reader, the LFA could reproducibly detect >3.6 ng ricin/mL (0.54 ng/test) in various 'white powder' samples and aerosol filter extracts. Because this assay does not discriminate among

Ricinus communis agglutinin (RCA) 60, RCA120, and ricin A chain, it can be used only as a qualitative screening assay when testing unknown samples. The test strips have also been evaluated by the EPA ETV Program for the detection of ricin in water samples. Reports and information associated with these evaluations are in the additional resource cited below.

Special Considerations: This LFA is listed as Tier I for solid, aerosol and water samples, and Tier II for all other environmental sample types. Crude preparations of ricin may also contain agglutinins that are unique to castor beans and that can cross-react in the immunoassays. A hook effect in the response of LFAs occurs when the amount of antigen in a sample overwhelms the amount of detector antibody present in the LFA. The resultant free antigen competes with the antigen-detector antibody complex for the capture antibody, which results in a decrease in the response and, under extreme conditions, can produce false-negative results. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative. False-negative results due to the hook effect were not observed with the commercial LFA evaluated, although a quantitative decrease in the response has been observed at high ricin concentrations. The incorporation of a serial dilution step in the sample protocol can eliminate such potential errors.

Source: Hodge, D.R., Prentice, K.W., Ramage, J.G., Prezioso, S., Gauthier, C., Swanson, T., Hastings, R., Basavanna, U., Datta, S., Sharma, S.K., Garber, E.A.E., Staab, A., Pettit, D., Drumgoole, R., Swaney, E., Estacio, P.L., Elder, I.A., Kovacs, G., Morse, B.S., Kellogg, R.B., Stanker, L., Morse, S. and Pillai, S.P. 2013. "Comprehensive Laboratory Evaluation of a Highly Specific Lateral Flow Assay for the Presumptive Identification of Ricin in Suspicious White Powders and Environmental Samples." *Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*. 11(4): 237-250.
https://www.ncbi.nlm.nih.gov/pubmed/24320219

Additional Resource: Environmental Technology Verification (ETV) Program Report. 2004. Anthrax, Botulinum Toxin, and Ricin Immunoassay Test Strips. Cincinnati, OH: EPA.
https://www.epa.gov/sites/default/files/2015-07/documents/etv-biothreat092104.pdf

8.2.15.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Ricin in various foods and beverages
Method Selected for: This method has been selected for presumptive analysis of ricin in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial antigen-capture ELISA detects antigens in samples by capturing them between a sandwich of antibodies. Positive and negative capture antibody reagents are applied to alternating wells of a 96-well plate, where they are passively adsorbed. Sample is then applied to the wells; if the target antigen is present in the sample, it will bind to the capture antibody. A detector antibody forms the top of the sandwich and binds to any bound antigen in the sample. The conjugate, to which the enzyme is covalently bound, is the third reagent added and binds to the detector antibody. The substrate, added after the conjugate, changes color in the presence of HRP. The amount of color change is directly proportional to the amount of HRP present, which correlates to the amount of ricin. Forty-eight samples can be processed in approximately 5 hours. The reference source reports an LOD of less than or equal to 0.02 µg/g. The additional resource provided below (ETV report 2004) describes the performance of this ELISA for water samples, and reports an LOD of 0.0075 mg/L.

Special Considerations: This method is listed as Tier II for presumptive analysis of ricin in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders, and

aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: Garber, E.A., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." *Journal of Food Protection*. 68(6): 1294-1301. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294

Additional Resource: James, R., Dinal, A., Willenberg, Z. and Riggs, K. Environmental Technology Verification (ETV) Report. 2004. "Anthrax, Botulinum Toxin, and Ricin Enzyme-Linked Immunosorbent Assay (ELISA)." Cincinnati, OH: EPA. https://archive.epa.gov/nrmrl/archive-etv/web/pdf/01_vr_elisa.pdf

8.2.15.3 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)
Method Developed for: Ricin in drinking water and particulate samples
Method Selected for: This method has been selected for presumptive analysis of ricin in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water and particulates.

Description of Method: This ECL-based immunoassay detects ricin in drinking water and particulate samples. After sample processing, samples are added to a 96-well plate with integrated anti-ricin capture antibody coated carbon electrodes and incubated. After incubation, detection antibodies with an ECL label are added to each well. An electrode potential is applied to the wells by an ECL instrument and light is generated. The light is captured through use of optics and a CCD camera on the instrument. Light emitted from each of the spots in the well is quantified by the instrument software.

Special Considerations: This method is listed as Tier I for presumptive analysis of ricin in particulate and drinking water samples and Tier II for presumptive analysis of ricin in all other environmental sample types. The sample preparation procedures described may facilitate analysis of other environmental sample types; however, further research is needed to verify their efficacy.

Source: U.S. EPA. August 2022. Protocol for Detection of Ricin Biotoxin in Environmental Samples During the Removal Phase of Response to a Contamination Incident. Cincinnati, Ohio. EPA/600/R-22/033A. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=355320&Lab=CESER

Additional Resource: Garber, E.A.E. and O'Brien, T.W. 2008. "Detection of Ricin in Food Using Electrochemiluminescence-Based Technology." *Journal of AOAC International*. 91(2): 376-382. https://www.ncbi.nlm.nih.gov/pubmed/18476351

8.2.15.4 Presumptive Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Ricinine in drinking water samples
Method Selected for: This method has been selected for presumptive analysis of ricin by ricinine detection in aerosol, solid, particulate and water samples. Ricinine, an alkaloid component of castor beans, is found in crude preparations of ricin and may be an indicator of ricin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involves sample extraction by SPE, followed by LC-MS-MS analysis, using an isocratic LC gradient and detection by ESI-MS-MS. Samples are combined with isotopically-labeled internal standards, and sample extracts are concentrated to dryness under nitrogen and heat, then adjusted to a 100-µL volume in HPLC-grade water. Accuracy and precision data are provided for application of the method to reagent water, finished ground water

and surface waters containing residual chlorine and/or chloramine. The method has DL of 0.09 µg/mL and an MRL of 0.50 µg/mL for ricinine. Storage stability was tested at 4°C for up to 28 days for ricinine (50 µg/mL in ground water and surface water containing either chlorine or monochloramine). Analyte percent recoveries at five hours ranged from 94.2% (± 2) to 110.4% (± 9.5); percent recoveries at 28 days ranged from 86% (± 10) to 92% (± 7).

Special Considerations: This method is listed as Tier I for presumptive analysis of ricin (as ricinine) in drinking water and Tier II for presumptive analysis of ricin (as ricinine) in all other environmental sample types. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. While ricinine can be used to indicate the presence of ricin, it can also be found alone, which is a limitation of this method.

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha-Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Knaack, J.S., Pittman, C.T., Wooten, J.V., Jacob, J.T, Magnuson, M., Silvestri, E. and Johnson, R.C. 2013. "Stability of ricinine, abrine, and alpha-amanitin in finished tap water." Analytical Methods. 20(5): 5804-5811.
http://pubs.rsc.org/en/content/articlelanding/2013/ay/c3ay40304a#!divAbstract

8.2.15.5 Confirmatory Analysis

Analytical Technique: Immunocapture / LC-MS-MS

Method Developed for: Ricin in beverages and tap water
Method Selected for: These procedures have been selected for confirmatory analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: MS is used in a two-pronged approach for detection and quantification of ricin: LC-MS-MS or MRM-MS for absolute quantification of toxin using isotope dilution MS, combined with an enzymatic assay and MALDI-TOF-MS detection to determine functional activity. Both approaches include an antibody capture step (polyclonal anti-ricin antibodies immobilized on magnetic beads) for ricin extraction. Quantitative analysis is achieved by trypsin digestion to generate peptides that are analyzed by LC-MS-MS and quantified relative to isotope internal peptide standards. Ricin can be quantified down to 10 fmol/mL in tap water, milk, apple juice and orange juice.

Special Considerations: These procedures are listed as Tier I for confirmatory analysis of ricin in drinking water and Tier II for confirmatory analysis of ricin in all other environmental sample types. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Some information regarding the analysis of ricin in supernatants obtained from wipe samples is provided in the additional resource cited below. The quantification assay and enzymatic activity assay can be run either in parallel (to return results faster when sample is plentiful), or the quantification can be performed on ricin-bound beads after the enzymatic activity has been assessed (when sample is limited). It should be noted that the antibody capture step does not distinguish between ricin A chain and intact ricin (both A and B chains).

Source: McGrath, S.C., Schieltz, D.M., McWilliams, L.G., Pirkle, J.L. and Barr, J.R. 2011. "Detection and Quantification of Ricin in Beverages Using Isotope Dilution Tandem Mass Spectrometry." *Analytical Chemistry*. 83: 2897-2905.
http://pubs.acs.org/doi/abs/10.1021/ac102571f

8.2.15.6 Analysis of Biological Activity

Analytical Technique: Immunocapture / MALDI-TOF-MS

Method Developed for: Ricin activity in beverages and tap water
Method Selected for: These procedures have been selected for biological activity analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: This method is an *in vitro* MALDI-TOF-MS-based activity assay that detects ricin-mediated depurination of synthetic substrates. Ricin is captured from a sample using a specific anti-ricin polyclonal antibody specific for B chain coupled to magnetic beads prior to assay. The magnetic bead/ricin complex is then added directly to the activity assay containing substrate and following incubation, the ratio of the peak areas of the product and the remaining unreacted substrate are determined by MALDI-TOF-MS for quantitative analysis. The source reference describes optimal assay parameters including use of a more efficient RNA substrate, assay buffer components, pH, and reaction temperature. In addition, optimization of the mass spectrometry analysis including MALDI matrix and sample preparation is described. With optimized parameters, the limit of detection of 0.2 ng/mL of ricin spiked in buffer and milk was accomplished. Improved assay reproducibility also made it possible to quantitatively detect active ricin with 3 orders of magnitude dynamic range.

Special Considerations: These procedures are listed as Tier I for analysis of the biological activity of ricin in drinking water and Tier II for analysis of the biological activity of ricin in all other environmental sample types, since non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. This may result in an aqueous matrix perhaps similar to matrices investigated in the source document. The deadenylase activity assay cannot distinguish between ricin A chain and intact ricin (both A and B chains); the antibody capture step is required to isolate the intact toxin prior to the activity assay.

Source: Wang, D., Baudys, J., Barr, J.R., and Kalb, S.R. 2016. "Improved Sensitivity for the Qualitative and Quantitative Analysis of Active Ricin by MALDI-TOF Mass Spectrometry." *Analytical Chemistry*. 88: 6867-6872. https://pubs.acs.org/doi/10.1021/acs.analchem.6b01486

8.2.16 Saxitoxins

CAS RNs: 35523-89-8 (STX) 64296-20-4 (NEO), 58911-04-9 (dcSTX), 68683-58-9 (dcNEOSTX), 143084-69-9 (doSTX), 77462-64-7 (GTX), 122075-86-9 (dcGTX)
Considered Variants: Saxitoxins (STX), Neosaxitoxins (NEO), Gonyautoxins (GTX)

Description: A suite of more than 50 structurally related neurotoxins produced by algae and cyanobacteria, including STX, NEO, GTX and decarbamoylsaxitoxin (dcSTX). Composed of 3,4-propinoperhydropurine tricyclic carbamates.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Method 2011.27	Presumptive	Receptor Binding Assay (RBA)	8.2.16.1
Toxicon. 2009. 54: 313-320	Presumptive	Immunoassay (ELISA)	8.2.16.2
Harmful Algae. 2016. 77-90	Presumptive	Immunoassay (ELISA)	8.2.16.3
J. Chromatogr. A. 2015. 1387: 1-12	Confirmatory	LC-MS-MS	8.2.16.4

8.2.16.1 Presumptive Analysis

Analytical Technique: RBA

Method Developed for: Determination of paralytic shellfish toxins (PSTs) in shellfish
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: An RBA is used to determine saxitoxins in shellfish tissue homogenates. The procedure is a competitive binding assay in which [^{3}H] STX competes with unlabeled STX standards or samples containing PSTs for a finite number of receptor sites in a rat brain membrane preparation. Tissue samples are extracted with 0.1 M hydrochloric acid at a pH of 3.0–4.0, followed by heating, cooling to room temperature, decantation and centrifugation to obtain clarified supernatant. Bound [^{3}H] STX is quantified by liquid scintillation counting. A standard curve is generated using unlabeled STX standards, which results in reduction in bound [^{3}H] STX that is proportional to the amount of unlabeled toxin. The concentration of STX in samples is determined in reference to the standard curve. Incubations are carried out in a microplate format to minimize sample handling and the amount of radioactivity used.

Special Considerations: This method is listed as Tier II for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. Samples must be extracted under acidic (pH 3–4) conditions to prevent toxin degradation. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: AOAC International. 2011. Official Method 2011.27: Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay, First Action.
http://www.eoma.aoac.org/methods/info.asp?ID=49771

8.2.16.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of PSTs in shellfish
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in aerosol, solid and particulate samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available ELISA kit is used for detection of saxitoxins in shellfish. The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Shellfish homogenates are heat extracted in 1% acetic acid and diluted prior to assay. When present in the sample, saxitoxin and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the saxitoxin present in the sample. The color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The kit allows the determination of 42 samples in duplicate, with a total analysis time of 60 minutes. The assay LOD for saxitoxin in shellfish homogenates is reported as 0.02 ppb (0.02 µg/L). Cross-reactivity of other PSTs (e.g., NEO, GTX 1-4) are considerably less than STX in this ELISA, and their presence may be underestimated in samples containing complex toxin profiles.

Special Considerations: The ELISA assay is listed as Tier II for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. The extraction procedure used for shellfish may be applicable to non-aqueous environmental sample types. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Costa, P.R., Baugh, K.A., Wright, B., RaLonde, R., Nance, S.L., Tatarenkova, N., Etheridge, S.M. and Lefebvre, K.A. 2009. “Comparative determination of paralytic shellfish

toxins (PSTs) using five different toxin detection methods in shellfish species collected in the Aleutian Islands, Alaska." *Toxicon*. 54(3): 313-320. http://www.sciencedirect.com/science/article/pii/S0041010109002402

8.2.16.3 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of PSTs in water
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available immunoassay (ELISA) is used to detect saxitoxins in water samples. The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Water samples are prepared by three successive freeze-thaw cycles followed by filtration prior to assay. Saxitoxin and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the saxitoxin present in the sample. The color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The kit allows the determination of 42 samples in duplicate, with a total analysis time of 60 minutes. The MRL reported for saxitoxin in water samples is 0.02 µg/L. Cross-reactivities of other PSTs (e.g., NEOSTX, GTX 1-4) are considerably less than STX in this ELISA.

Special Considerations: The ELISA method is listed as Tier I for presumptive analysis of saxitoxins in water samples. Non-aqueous samples require extraction prior to assay (see Section 8.2.16.2). Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Loftin, K.A., Graham, J.L., Hilborn, E.D., Lehmann, S.C., Meyer, M.T., Dietze, J.E. and Griffith, C.B. 2016. "Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007." *Harmful Algae*. 56: 77-90. http://www.sciencedirect.com/science/article/pii/S1568988315300883

8.2.16.4 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of saxitoxins (STX, NEO, GTX, dcGTX, dcSTX) and saxitoxin analogues in shellfish
Method Selected for: These procedures have been selected for confirmatory analysis of saxitoxins and analogues in aerosol, solid, particulate and water samples. Modification and further research are needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method uses multiple analogues of saxitoxin in shellfish are detected using LC-MS-MS. The method includes a single dispersive extraction of shellfish homogenates followed by SPE using graphitized carbon cartridges for sample cleanup and LC-MS-MS, with a hydrophilic interaction liquid chromatography (HILIC) column, for analysis. Validation study results included in Turner et al. (2015) are provided for specificity, linearity, recovery, repeatability, and within-laboratory reproducibility. LOD and LOQ were significantly improved in comparison to other currently available fluorescence-based detection methods.

Special Considerations: This method is listed as Tier II for confirmatory analysis of saxitoxin in aerosol, solid, particulate and water samples. The sample extraction and cleanup (SPE)

procedures described for shellfish homogenates may be suited for other sample types with slight modifications. For example, relatively clean samples (e.g., drinking water and liquid samples) may not require extensive extraction procedures prior to sample cleanup and analyte concentration using SPE. In addition, adaptation of online SPE in conjunction with LC-MS-MS, as reported by Bragg et al. (2015) for STX and NEO, and by Coleman et al. (2016) for tetrodotoxin analysis, may provide additional streamlining and high-throughput capacity for saxitoxin analysis.

Source: Boundy, M.J., Selwood, A.I., Harwood, D.T., McNabb, P.S. and Turner, A.D. 2015. "Development of a sensitive and selective liquid chromatography–mass spectrometry method for high-throughput analysis of paralytic shellfish toxins using graphitized carbon solid phase extraction." *Journal of Chromatography A*. 1387: 1-12. http://www.sciencedirect.com/science/article/pii/S0021967315001995

Additional Resources:

- Turner, A.D., McNabb, P.S., Harwood, A.J. and Boundy, M.J. 2015. "Single-Laboratory Validation of a Multitoxin Ultra-Performance LC-Hydrophilic Interaction LC-MS/MS Method for Quantitation of Paralytic Shellfish Toxins in Bivalve Shellfish." *Journal of AOAC International*. 98(3): 609-621. https://doi.org/10.5740/jaoacint.14-275
- Bragg, W.A., Lemire, S.W., Coleman, R.M., Hamelin, E. I. and Johnson, R.C. 2015. "Detection of human exposure to saxitoxin and neosaxitoxin in urine by online-solid phase extraction-liquid chromatography-tandem mass spectrometry." *Toxicon*. 99: 118-124. https://www.ncbi.nlm.nih.gov/pubmed/25817003
- Coleman, R., Lemire, S.W., Bragg, W., Garrett, A., Ojeda-Torres, G., Hamelin, E., Johnson, R. C. and Thomas, J. 2016. "Development and validation of a high-throughput online solid phase extraction-liquid chromatography-tandem mass spectrometry method for the detection of tetrodotoxin in human urine." *Toxicon*. 119: 64-71. http://www.sciencedirect.com/science/article/pii/S0041010116301386

8.2.17 Shiga and Shiga-like Toxins (Stx)

CAS RN: 75757-64-1 (Stx)
Considered Variants: Stx-1a, 1c, 1d and 1e, Stx-2a to 2g
Description: Protein produced by *Shigella dysenteriae* and the shiga toxin-producing *Escherichia coli* (STEC). Composed of one ~32 kDa A chain and five 7.7 kDa B chains.

Selected Methods	Analysis Type	Analytical Technique	Section
Austin Immunology. 2016. 1(2), id1007: 1−7	Presumptive	Immunoassay (ELISA)	8.2.17.1
Analytical Chemistry. 2014. 86: 4698−4706	Confirmatory	LC-MS-MS	8.2.17.2

8.2.17.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Shiga-like toxins (Stx-1 and Stx-2) in foods and bacterial enrichment broth
Method Selected for: These procedures have been selected for presumptive analysis of Shiga and Shiga-like toxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for these sample types.

Description of Method: Shiga-like toxins (Stx-1, including 4 Stx-1a, 1c, 1d and 1e four subtypes and Stx-2, including Stx-2a to 2g seven subtypes) are produced by various STEC. Two commercial ELISA kits (Stx-1 ELISA and Stx-2 ELISA) are used to detect these toxins, using

type-specific, polyclonal and mAbs to differentiate between the Stx-1 and Stx-2 toxin types. Samples are incubated with a medium that encourages *E. coli* (EC) growth and Stx production. If Stx-1or Stx-2 is present, it is bound by an immobilized polyclonal antibody on the wells of a microtiter plate. After a washing step, a mixture of mAbs is added which binds to the Stx-1 or Stx-2. A second washing step is followed by the addition of an HRP-labeled antibody that binds to the existing antigen/antibody complex in the wells. After a final wash and addition of substrate solution, a color signal is generated. The color intensity is evaluated using an ELISA plate reader and is proportional to the amount of Stx-1 or Stx-2 present. The result is compared to a known value to determine whether the sample is positive or below the limit of Stx-1 or Stx-2 detection (25 pg/mL). The additional source citation (below) describes the evaluation of these two ELISA kits for STEC-inoculated and enriched ground beef, romaine lettuce, recreational pond water, and pasteurized milk samples. Results indicate that Stx-1 and Stx-2 were readily detected and distinguished for all tested sample types.

Special Considerations: This assay is listed as Tier I for non-drinking water samples and a Tier II for presumptive analysis of Shiga-like toxins in aerosol, solid, particulate and drinking water samples. Appropriate standards are available. Sample preparation procedures used for foods and bacterial broth suggest that similar aqueous extraction procedures may be applicable to environmental samples. The Stx-1 ELISA may also be applicable for analysis of Stx produced by *Shigella* bacteria.

Source: Kong, Q., Patfield, S., Skinner, C., Stanker, L.H., Gehring, A. G., Fratamico, P.M., Rubio, F., Qi, W., and He, X. 2016. "Validation of Two New Immunoassays for Sensitive Detection of a Broad Range of Shiga Toxins." *Austin Immunology*. 1(2), id 1007: 1-7. https://austinpublishinggroup.com/austin-immunology/fulltext/ai-v1-id1007.php

Additional Resource: Gehring, A.G., Fratamico, P.M., Lee, J., Ruth, L.E., He, X., He, Y., Paoli, G.C., Stanker, L.H. and Rubio, F.M. 2017. "Evaluation of ELISA Tests Specific for Shiga Toxin 1 and 2 in Food and Water Samples." *Food Control*. 77: 145-149. http://dx.doi.org/10.1016/j.foodcont.2017.02.003

8.2.17.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Stx-1 and Stx-2 in plasma and enrichment broth
Method Selected for: These procedures have been selected for confirmatory analysis of Shiga and Shiga-like toxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for these sample types.

Description of Method: This MRM method is based on analyzing conserved peptides, derived from the tryptic digestion of the toxin B subunits. Stable isotope-labeled analogues are prepared and used as internal standards to identify and quantify these characteristic peptides. The method detects and quantifies Shiga toxins (Stx) and Shiga-like toxins type 1 (Stx-1) and type 2 (Stx-2), and also distinguishes among most of the known Stx-1 and Stx-2 subtypes. The LOD for digested pure standards is approximately 10 attomole range/injection, which corresponds to a concentration of 1.7 femtomol/mL. Samples and standards are reduced using dithiothreitol, then alkylated using iodoacetamide, and finally subjected to proteolysis by the addition of a trypsin solution and incubation at 37°C for 16 hours. Digested samples are filtered through a 10,000 molecular weight cut-off (MWCO) filter. For quantitative analysis, an aliquot of filter-sterilized sample is digested with trypsin and a fixed amount of the appropriate trypsin digested ^{15}N-labled internal standard is added. An MS equipped with a linear ion trap and a nanoelectrospray source was used to perform LC-MS-MS, operated in MRM mode, and alternating between detection of the nine peptides and the corresponding ^{15}N-labeled internal standards. The additional source citation (see below) provides a more rapid (3-hour) approach to reduce/alkylate and trypsin digest serum samples, resulting in equivalent or improved detection compared to the procedure described above.

Special Considerations: This method is listed as Tier II for confirmatory analysis of Shiga-like toxins in aerosol, solid, particulate and water samples. Appropriate standards are available. Sample preparation procedures used for plasma and bacterial broth suggest that aqueous extraction may be applicable to environmental samples. Matrix effects were observed when dilute samples were digested in buffer, Luria broth, or mouse plasma (LOD ~30 attomol/injection = 5 femtomol/mL). This method may also be applicable for analysis of Stx produced by *Shigella* bacteria, using an appropriate standard.

Source: Silva, C.J., Erickson-Beltran, M.L., Skinner, C.B., Dynin, I., Hui, C., Patfield, S.A., Carter, J.M. and He, X. 2014. "Safe and Effective Means of Detecting and Quantitating Shiga-Like Toxins in Attomole Amounts." *Analytical Chemistry*. 86(10): 4698-4706. http://pubs.acs.org/doi/abs/10.1021/ac402930r

Additional Resource: Silva, C.J., Erickson-Beltran, M.L., Skinner, C.B., Patfield, S.A. and He, X. 2015. "Mass Spectrometry-Based Method of Detecting and Distinguishing Type 1 and Type 2 Shiga-Like Toxins in Human Serum." *Toxins*. 7(12): 5236-5253. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4690125/

8.2.18 Staphylococcal Enterotoxins (SETs)

CAS RNs: 37337-57-8 (staphylococcal enterotoxin type A [SEA]), 39424-53-8 (staphylococcal enterotoxin type B [SEB]), 39424-54-9 (staphylococcal enterotoxin type C [SEC]), 12788-99-7 (staphylococcal enterotoxin type D [SED]), (staphylococcal enterotoxin type E [SEE])
Considered Variants: SEA − SEE

Description: Heat stable, basic, single-chain proteins with molecular weights of 26,000 to 29,000 kDa, produced by certain *Staphylococcus* strains.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Official Method 2007.06	Presumptive	Immunoassay (Enzyme-linked fluorescent immunoassay [ELFA])	8.2.18.1
Journal of AOAC International. 2014. 97(3): 862-867	Presumptive	Immunoassay (ECL)	8.2.18.2
Letters in Applied Microbiology. 2011. 52: 468-474	Confirmatory	Immunoassay (ELISA)	8.2.18.3

8.2.18.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELFA)

Method Developed for: Staphylococcal enterotoxins in selected foods
Method Selected for: This method has been selected for presumptive analysis of SETs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial test is an ELFA used with automated instrumentation for the specific detection of SEA − SEE. The solid-phase-receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips. The instrument performs all of the assay steps automatically. The user places the sample extract into the reagent strip, and the sample is cycled in and out of the SPR for a specific length of time. SET present in the sample will bind to the anti-SET mAbs, which are coated on the interior of the SPR. Unbound sample components are washed away. Alkaline phosphatase-labeled antibodies are cycled in and out of the SPR and will bind to any SET captured on the SPR wall. Further wash steps remove unbound conjugate. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and

out of the SPR. The bound enzyme conjugate catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The test value is calculated by the instrument and is equal to the sample relative fluorescence value (RFV)/standard RFV. A "negative" result has a test value less than the threshold (0.13), indicating that the sample does not contain SET or contains SET at a concentration below the DL. A "positive" result has a test value equal to or greater than the threshold and indicates that the sample is contaminated with SET. DLs for SETs in various foods range from 0.25 ng/g to 0.5 ng/g (solids) or ng/mL (liquids).

Special Considerations: These procedures are listed as Tier II for presumptive analysis of staphylococcal neurotoxins in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: AOAC International. 2007. "Method 2007.06: "VIDAS SET 2 for Detection of Staphylococcal Enterotoxins in Selected Foods" *Journal of AOAC International*. 91: 164. http://www.aoacofficialmethod.org/index.php?main_page=product_info&cPath=1&products_id=1827

8.2.18.2 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)

Method Developed for: Determination of SEB in foods
Method Selected for: These procedures have been selected for presumptive analysis of SEB in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial ECL immunoassay is used with automated instrumentation for the specific detection of SEB. The source reference describes a comparison of its use to ELISA and LFD assays, in conjunction with an outbreak of staphylococcal food poisoning, and notes demonstrated cross-reactivity with SED present in the food samples as well as interferences from the food matrices tested.

Special Considerations: These procedures are listed as Tier III for presumptive analysis of SEB in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Please consult the technical contacts listed in Section 4.0 for information regarding the status and availability of assay reagents and analytical instrumentation.

Source: Tallent, S. M., Hait, J., and Bennet, R., J. 2014. "Staphylococcal Enterotoxin B-Specific Electrochemiluminescence and Lateral Flow Device Assays Cross-React with Staphylococcal Enterotoxin D." *Journal of AOAC International*. 97(3): 862-867. https://doi.org/10.5740/jaoacint.13-198

8.2.18.3 Confirmatory Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of SETs in naturally contaminated cheese
Method Selected for: These procedures have been selected for confirmatory analysis of SETs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available sandwich type enzyme immunoassay (ELISA) detects the combined SET types SEA through SEE. The surface of a microtitre plate is coated with specific purified antibodies that bind enterotoxins. The immobilized toxins are then bound by a mixture of toxin-specific detector antibodies (conjugated to biotin) forming a

sandwich complex (antibody–antigen–antibody complex). Addition of specific antibodies forms a sandwich complex (antibody–antigen–antibody). The presence of enterotoxins is revealed by adding an enzyme substrate⁄chromogen solution containing tetramethylbenzidine, which results in a blue color in the presence of SETs. Addition of a sulphuric acid solution leads to a color change from blue to yellow, allowing for the confirmation of the presence of SEs using a spectrophotometer at a double wavelength of 450⁄630 nm. The sample is considered to be contaminated by SEs if the absorbance test value is greater than or equal to the threshold value (negative control value plus 0.15). Solid food samples (e.g., cheese) are homogenized, centrifuged and concentrated by dialysis prior to assay. DLs reported for cheese samples range from 0.012 ng/g (SEA) to 0.05 ng/g (SED).

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of SETs in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. All samples may require a concentration step (e.g., dialysis) prior to assay.

Source: Ostyn, A., Fuillier, F., Prufer, A.L., Messio, S., Krys, S., Lombard, B. and Hennekinne, J.A. 2011. "Intra-laboratory validation of the Ridascreen® SET Total kit for detection staphylococcal enterotoxins SEA to SEE in cheese." *Letters in Applied Microbiology*. 52(5): 468-474. http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2011.03025.x/abstract

8.2.19 T-2 Mycotoxin

CAS RN: 21259-20-1 (T-2), 26934-87-2 (HT-2)
Considered Variants: T-2 and HT-2

Description: Trichothecene toxins produced by *Fusarium* spp.

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of Food Protection. 2005. 68(6): 1294-1301	Presumptive	Immunoassay (ELISA)	8.2.19.1
Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422-1428	Confirmatory	LC-MS	8.2.19.2

8.2.19.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: T-2 mycotoxin in food and beverages
Method Selected for: These procedures have been selected for presumptive analysis of α-amanitin (see Section 8.2.3.1) and T-2 toxin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available ELISA is used to detect T-2 mycotoxin at levels below those described as a health concern in food samples. Solid food samples are prepared by extracting the sample with methanol/water followed by dilution with phosphate-buffered saline. Liquid beverage samples are prepared by dilution in sodium phosphate buffer. The prepared samples are analyzed using commercially obtained ELISA kits according to the manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 26 minutes of incubation at 37°C.

Special Considerations: This assay is listed as Tier II for presumptive analysis of T-2 mycotoxins in aerosol, solid, particulate and water samples. Non-aqueous samples will require extraction prior to analysis. The ELISA kit successfully detects T-2 toxin at targeted levels of 0.2 µg/g; the immunoassay for T-2 toxin, however, shows variable background responses up to 0.1 µg/g. Detection thresholds of 0.2 µg/g for T-2 toxin avoided the background problems but were

low enough to allow detection at concentrations below those associated with serious health effects. The additional resource citation (Tima et al. 2016) describes the application of this ELISA for monitoring swine feedstuff for T-2 mycotoxin. The LOD for T-2 mycotoxin in this study was 12 µg/kg.

Source: Garber, E.A.E., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." *Journal of Food Protection*. 68(6): 1294-1301. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294?=

Additional Resource: Tima, H., Rácz, A., Guld, Z., Mohácsi-Farkas, C. and Kiskó, G. 2016. "Deoxynivalenol, zearalenone and T-2 in grain based swine feed in Hungary." *Food Additives & Contaminants: Part B*. 9(4):275-280. DOI: 10.1080/19393210.2016.1213318. http://dx.doi.org/10.1080/19393210.2016.1213318

8.2.19.2 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: Mycotoxins in food
Method Selected for: These procedures have been selected for confirmatory analysis of T-2 and HT-2 mycotoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A LC-TOF-MS with atmospheric pressure chemical ionization (APCI) and time-of-flight (TOF)-MS with a real-time reference mass correction is used for simultaneous determination of *Fusarium* mycotoxins (including T-2 and HT-2 mycotoxins) in food. Samples are extracted and centrifuged, and the supernatant is applied to a MultiSep #226 column for cleanup. Prepared samples are separated by reversed-phase LC and detected by APCI-MS. LODs range from 0.1 to 0.3 ng/g and 0.5 to 0.9 ng/g for T-2 and HT-2 in analyzed foodstuffs, respectively. The additional resource citation below (Garcia-Moraleja et al. 2015) describes a liquid-liquid extraction procedure using either triple quadrupole or ion trap LC-MS-MS for determination of various mycotoxins in brewed coffee.

Special Considerations: This method is listed as Tier II for confirmatory analysis of T-2 mycotoxins in aerosol, solid, particulate and water samples. The procedures described may need to be modified for water, soil, aerosol and particulate samples. It may be possible to analyze relatively clean water samples by direct injection into an LC-MS-MS. Dirty water samples or water samples with low concentrations of toxin may require cleanup and concentration.

Source: Tanaka, H., Takino, M., Sugita-Konishi, Y. and Tanaka, T. 2006. "Development of Liquid Chromatography/Time-of-Flight Mass Spectrometric Method for the Simultaneous Determination of Trichothecenes, Zearalenone, and Aflatoxins in Foodstuffs." *Rapid Communications in Mass Spectrometry*. 20(9): 1422-1428. http://onlinelibrary.wiley.com/doi/10.1002/rcm.2460/abstract

Additional Resource: Garcia-Moraleja, A., Font, G., Manes, J. and Ferrer, E. 2015. "Development of a new method for the simultaneous determination of 21 mycotoxins in coffee beverages by liquid chromatography tandem mass spectrometry." *Food Research International*. 72: 247-255. http://www.sciencedirect.com/science/article/pii/S0963996915001167

8.2.20 Tetrodotoxin (TTX)

CAS RN: 9014-39-5
Considered Variants: NA

Description: Neurotoxin produced by certain infecting or symbiotic bacteria like *Pseudomonas*

and *Vibrio,* found in animals from the order Tetraodontiformes, which includes pufferfish, porcupinefish, ocean sunfish, and triggerfish. Composed of heterocyclic heat-stable (except in alkaline environments) and water-soluble neurotoxin.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Official Method 2011.27. 2011	Presumptive	RBA	8.2.20.1
Journal of AOAC International. 2017. 100(5): 1469-1482	Confirmatory	LC-MS-MS	8.2.20.2

8.2.20.1 Presumptive Analysis

Analytical Technique: RBA

Method Developed for: PSTs in shellfish
Method Selected for: This method has been selected for confirmatory analysis of TTX in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This assay is used for determination of PSTs in shellfish tissue homogenates. The procedure is a competitive binding assay in which [^{3}H] STX competes with unlabeled standards (TTX) or PSTs in samples for a finite number of receptor sites in a rat brain membrane preparation. Tissue samples are extracted with 0.1 M hydrochloric acid at a pH of 3.0–4.0, followed by heating, cooling to room temperature, decantation and centrifugation to obtain clarified supernatant. Unbound [^{3}H] STX is removed by filtration and bound [^{3}H] STX is quantified by liquid scintillation counting. A standard curve is generated using unlabeled TTX standards, which results in reduction in bound [^{3}H] STX that is proportional to the amount of unlabeled toxin. The concentration of TTX in samples is determined in reference to the standard curve. Incubations are carried out in a microplate format to minimize sample handling and the amount of radioactivity used.

Special Considerations: This method is listed as Tier II for presumptive analysis of TTX in aerosol, solid, particulate and water samples. Samples must be extracted under acidic (pH 3–4) conditions to prevent toxin degradation. This method was originally developed for STX but can be used to quantify TTX in samples when TTX standards are used to develop a standard curve since both toxins compete for the same receptor.

Source: AOAC International. 2011. Official Method 2011.27: Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay, First Action.
http://www.eoma.aoac.org/methods/info.asp?ID=49771

8.2.20.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Analysis of TTX in common mussels and Pacific oysters
Method Selected for: These procedures have been selected for confirmatory analysis of TTX in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method consists of a single-step dispersive sample extraction in 1% acetic acid, followed by a carbon SPE cleanup step, dilution, and analysis by HILIC-MS-MS. The method was developed for the quantitation of TTX, as well as the associated analogs 4-epi-TTX; 5,6,11-trideoxy TTX; 11-nor TTX-6-ol; 5-deoxy TTX; and 4,9-anhydro TTX. The source reference reports method performance parameters for both mussel and Pacific oyster sample types, including specificity, linearity, LODs (approximately 0.25 µg/kg), LOQs (0.79 and 0.76 µg/kg for mussels and Pacific oysters, respectively), and reporting limits (set to 2 µg/kg).

Special Considerations: This method is listed as Tier II for confirmatory analysis of TTX in aerosol, solid, particulate and water samples. The procedures described for shellfish extraction and cleanup may need to be modified for these sample types.

Source: Turner, A.D., Boundy, M.J., Rapkova, M.D. 2017. "Development and Single-Laboratory Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for Quantitation of Tetrodotoxin in Mussels and Oysters." *Journal of AOAC International*. 100(5): 1469-1482. https://doi.org/10.5740/jaoacint.17-0017

8.2.21 Zearalenone

CAS RN: 17924-92-4
Considered Variants: NA

Description: Zearalenone is a phenolic resorcyclic lactone produced by various *Fusarium* spp.

Selected Methods	Analysis Type*	Analytical Technique	Section
Journal of Agricultural and Food Chemistry. 2017. 65(33): 7138-7152	Confirmatory	LC-MS-MS	8.2.21.1

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

8.2.21.1 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins (including aflatoxins, deoxynivalenol, fumonisin, ochratoxin A and zearalenone) in corn, peanut butter and wheat flour
Method Selected for: These procedures have been selected for confirmatory analyses of zearalenone in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The source reference describes a collaborative laboratory study to evaluate an LC-MS-MS procedure using commercially available ^{13}C-labeled internal standards for simultaneous detection and quantification of multiple mycotoxins. The method described can be used to detect and quantify mycotoxins including: aflatoxins; deoxynivalenol; fumonisins B1, B2, and B3; ochratoxin A; and zearalenone. Procedures for sample fortification, extraction, filtration and centrifugation are described in addition to LC-MS-MS conditions and parameters for various platforms used by laboratories participating in the study. The ranges of analytical performance for the six laboratories depended on LC-MS instrument conditions (column injection volume, flow rate, etc.). Average recoveries of the participating laboratories were in the range of 90−110%, with repeatability RSD_r (within laboratory) < 10% and reproducibility RSD_R (among laboratories) < 15%. LOQ range for zearalenone was 0.5−1.0 ng/mL.

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of zearalenone in aerosol, solid, particulate and water samples. The sample preparation procedures described for food/feed (extraction with acetonitrile/water, centrifugation, and filtration) may be applicable to environmental samples.

Source: Zhang, K., Schaab, M.R., Southwood, G., Tor, E.R., Aston, L.S., Song, W., Eitzer, B., Majumdar, S., Lapainis, T., Mai, H., Tran, K., El-Demerdash, A., Vega, V., Cai, Y., Wong, J.W., Krynitsky, A.J. and Begley, T.H. 2017. "Collaborative Study: Determination of Mycotoxins in Corn, Peanut Butter, and Wheat Flour Using Stable Isotope Dilution Assay (SIDA) and Liquid Chromatography−Tandem Mass Spectrometry (LC-MS/MS)." *Journal of Agricultural and Food Chemistry*. 65(33): 7138-7152. https://doi.org/10.1021/acs.jafc.6b04872

Section 9.0: Conclusions

SAM is intended for use by EPA and EPA-contracted and -subcontracted laboratories and can also be used by other agencies and laboratory networks, such as the ICLN, which includes the ERLN and WLA. The information provided in this document also can be found on the SAM webpage, which provides a searchable query tool for users to access supporting information regarding selected methods.

The primary objective of SAM efforts is to identify appropriate methods that facilitate data comparability by providing existing, documented techniques, and consistent and valid analytical results. The methods selected for each analyte/sample type combination were deemed the most appropriate, and broadly applicable of available methods by work groups consisting of technical experts in each field. The selected methods are subject to change following further research to improve methods or following the development of new methods, and the contacts listed in Section 4.0 encourage the scientific community to inform them of any such method improvements.

Since publication of Revision 1.0 in September 2004, EPA's HSRP has continued to convene technical work groups to evaluate and, if necessary, update the analytes and methods that are listed in SAM. This current revision (2022) includes the addition of new analytes to the chemical, radiochemical and biotoxin technical sections (Sections 5.0, 6.0 and 8.0, respectively); several new methods selected for chemical, radiochemical, pathogen and biotoxin analytes; the addition of limestone as a sample type for radiochemicals in outdoor infrastructure and building materials; and the combination of drinking water and post-decontamination wastewater into a single water sample matrix for pathogens. Details regarding changes that have been incorporated into each revision are provided in Attachment 1.

Appendix A: Selected Chemical Methods

SAM 2022 — Appendix A: Selected Chemical Methods

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected chemical methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate quality control. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: Analyte/sample type is a target of the method(s). Data are available for all aspects of method performance and quality control measures supporting its use for analysis of environmental samples following a contamination incident. Evaluation and/or use of the method(s) in multiple laboratories indicate that the method can be implemented with no additional modifications for the analyte/sample type.

Tier II: (1) The analyte/sample type is a target of the method(s) and the method(s) has been evaluated for the analyte/sample type by one or more laboratories, or (2) the analyte/sample type is not a target of the method(s), but the method(s) has been used by laboratories to address the analyte/sample type. In either case, available data and/or information indicate that modifications will likely be needed for use of the method(s) to address the analyte/sample type (e.g., due to potential interferences, alternate matrices, the need to address different DQOs).

Tier III: The analyte/sample type is not a target of the method(s), and/or no reliable data supporting the method's fitness for its intended use are available. Data from other analytes or sample types, however, suggest that the method(s), with significant modification, may be applicable.

Notes:

- **The column headings listed in this Appendix are defined in Section 5.0. Summaries of and access to each method cited are provided in Section 5.2 (see Table 5-1 to locate a specific method summary).**
- **Some but not all of the analyte degradation products are included in this list. Method users should be aware of potential by-products and degradation products when performing analyses to identify and quantify specific target analytes.**

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
A-230 (Methyl-[1-(diethylamino)ethylidene]-phosphonamidofluoridate)	2387496-12-8	GC-MS	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	TO-17 (EPA ORD)	*III	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II
A-232 (Methyl-[1-(diethylamino)ethylidene]-phosphoramidofluoridate)	2387496-04-8	GC-MS	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	TO-17 (EPA ORD)	*III	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II
A-234 (Ethyl N-[(1E)-1-(diethylamino) ethylidene]-phosphoramidofluoridate)	2387496-06-0	GC-MS	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II	TO-17 (EPA ORD)	*III	SOP L-P-107, Rev.3 (EPA PHILIS) SOP L-A-507, Rev.3 (EPA PHILIS)	*II
Acephate	30560-19-1	LC-MS-MS	Adapted from J.Env.Sci. Health (2014) 49: 23-34 538 (EPA OW)	II	538 (EPA OW)	II	538 (EPA OW)	I	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III
Acrylamide	79-06-1	HPLC-UV	Water extraction 8316 (EPA SW-846)	III	8316 (EPA SW-846)	II	8316 (EPA SW-846)	II	PV2004 (OSHA)	I	3570/8290A Appendix A (EPA SW-846) 8316 (EPA SW-846)	III
Acrylonitrile	107-13-1	HPLC-UV / GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	II	524.2[1] (EPA OW)	II	524.2[1] (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846) 8260D (EPA SW-846)	III

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Aldicarb (Temik)	116-06-3	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Aldicarb sulfone	1646-88-4	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Aldicarb sulfoxide	1646-87-3	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	III	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Allyl alcohol	107-18-6	GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	TO-15[2] (EPA ORD)	III	Not of concern	NA
4-Aminopyridine	504-24-5	HPLC-UV	8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	III	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	III
Ammonia	7664-41-7	Visible spectrophotometry / IC	Not of concern**	NA	4500-NH_3 B (SM) 4500-NH_3 G (SM)	I	350.1 (EPA OW)	I	6016 (NIOSH)	I	Not of concern**	NA
Ammonium metavanadate (analyze as total vanadium)	7803-55-6	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Arsenic, Total	7440-38-2	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Arsenic trioxide (analyze as total arsenic)	1327-53-3	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	GFAA / ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	6001 (NIOSH)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Asbestos	1332-21-4	TEM	D5755-09(e1) (soft surfaces-microvac) (ASTM)	III	Not of concern**	NA	Not of concern**	NA	10312:1995 (ISO)	I	D6480-19 (hard surfaces-wipes) (ASTM)	I
Boron trifluoride	7637-07-2	ISE	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	ID216SG (OSHA)	I	Not of concern**	NA

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Brodifacoum	56073-10-0	LC-MS-MS	3541/3545A (EPA SW-846) D7644-16 (ASTM)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) D7644-16 (ASTM)	III
Bromadiolone	28772-56-7	LC-MS-MS	3541/3545A (EPA SW-846) D7644-16 (ASTM)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) D7644-16 (ASTM)	III
BZ [Quinuclidinyl benzilate]	6581-06-2	HPLC-UV / LC-MS-MS	3541/3545A (EPA SW-846) Adapted from J. Chromatogr. B (2008) 874: 42-50	III	Adapted from J. Chromatogr. B (2008) 874: 42-50	III	Adapted from J. Chromatogr. B (2008) 874: 42-50	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846) Adapted from J. Chromatogr. B (2008) 874: 42-50	III
Calcium arsenate (analyze as total arsenic)	7778-44-1	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Carbofuran (Furadan)	1563-66-2	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Carbon disulfide	75-15-0	GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	I	5030C (EPA SW-846) 8260D (EPA SW-846)	I	524.2[1] (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern**	NA
Carfentanil	59708-52-0	LC-MS-MS	3541/3545A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	Not of concern**	NA	PHILIS SOP L-A-309 Rev. 0 / L-A-310 Rev. 1	III
Chlorfenvinphos	470-90-6	GC-MS	EPA/600/R-16/114	II	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Chlorine	7782-50-5	Visible spectrophotometry	Not of concern**	NA	4500-Cl G (SM)	I	4500-Cl G (SM)	I	Adapted from Analyst (1999) 124(12): 1853-1857 4500-Cl G (SM)	II	Not of concern**	NA
2-Chloroethanol	107-07-3	GC-MS / GC-FID	5035A (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	2513 (NIOSH)	I	Not of concern**	NA

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
3-Chloro-1,2-propanediol	96-24-2	GC-MS	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	TO-10A[4] (EPA ORD)	III	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	III
Chloropicrin	76-06-2	GC-MS / GC-ECD	EPA/600/R-16/114[16]	II	551.1 (EPA OW)	I	551.1 (EPA OW)	I	PV2103 (OSHA)	I	EPA/600/R-16/114[16]	II
Chlorosarin	1445-76-7	GC-MS	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	TO-17[4] (EPA ORD)	*III	EPA/600/R-16/115	*III
Chlorosoman	7040-57-5	GC-MS	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	TO-17[4] (EPA ORD)	*III	EPA/600/R-16/115	*III
Chlorovinyl arsonic acid (CVAOA) (degradation product of Lewisite)	64038-44-4	LC-MS-MS / ICP-AES / ICP-MS	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	IO-3.1[6] (EPA ORD) / IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*II
2-Chlorovinylarsonous acid (CVAA) (degradation product of Lewisite)	85090-33-1	LC-MS-MS / ICP-AES / ICP-MS	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	IO-3.1[6] (EPA ORD) / IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*II
Chlorpyrifos	2921-88-2	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	II	525.2[7] (EPA OW)	II	TO-10A (EPA ORD)	I	EPA/600/R-16/114	II
Chlorpyrifos oxon	5598-15-2	GC-MS / LC-MS-MS	EPA/600/R-16/114	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Crimidine	535-89-7	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	II	EPA/600/R-16/114	II	Not of concern**	NA	EPA/600/R-16/114	II
Cyanide, Amenable to chlorination	NA	Visible spectrophotometry	3135.2I (EPA RLAB)	I	3135.2I[8] (EPA RLAB)	I	3135.2I[8] (EPA RLAB)	I	Not of concern**	NA	3135.2I (EPA RLAB)	III
Cyanide, Total	57-12-5	Visible spectrophotometry	ISM02.3 CN (EPA CLP)	I	ISM02.3 CN[9] (EPA CLP)	I	335.4 (EPA OW)	I	6010 (NIOSH)	I	ISM02.3 CN (EPA CLP)	III
Cyanogen chloride	506-77-4	GC-MS/GC-ECD	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	TO-15 (EPA ORD)	III	Not of concern**	NA
Cyclohexyl sarin (GF)	329-99-7	GC-MS	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	TO-17 (EPA ORD)	II	EPA/600/R-16/115	*I

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
1,2-Dichloroethane (degradation product of HD)	107-06-2	GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	I	5030C (EPA SW-846) 8260D (EPA SW-846)	I	524.2[1] (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern**	NA
Dichlorvos	62-73-7	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	I	525.2[7] (EPA OW)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114	II
Dicrotophos	141-66-2	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	I	3535A (EPA SW-846) 8270E (EPA SW-846)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114	II
Diesel range organics	NA	GC-FID	3541/3545A (EPA SW-846) 8015D (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8015D (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8015D (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8015D (EPA SW-846)	I
Diisopropyl methylphosphonate (DIMP) (degradation product of GB)	1445-75-6	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	538 (EPA OW)	I	TO-10A[4] (EPA ORD)	III	EPA/600/R-13/224	II
Dimethylphosphite	868-85-9	GC-MS	EPA/600/R-16/114	II	Not of concern**	NA	Not of concern**	NA	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	III	D7597-16 (ASTM)	III	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224	III
Diphacinone	82-66-6	LC-MS-MS	3541/3545A (EPA SW-846) D7644-16 (ASTM)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) D7644-16 (ASTM)	III
Disulfoton	298-04-4	GC-MS / GC-FPD	EPA/600/R-16/114	II	525.2[7,10] (EPA OW)	II	525.2[7,10] (EPA OW)	II	5600 (NIOSH)	I	EPA/600/R-16/114	II
Disulfoton sulfone oxon	2496-91-5	GC-MS / GC-FPD	EPA/600/R-16/114	III	525.2[7,10] (EPA OW)	III	525.2[7,10] (EPA OW)	III	5600 (NIOSH)	III	EPA/600/R-16/114	III
Disulfoton sulfoxide	2497-07-6	GC-MS / GC-FPD	EPA/600/R-16/114	III	525.2[7,10] (EPA OW)	II	525.2[7,10] (EPA OW)	II	5600 (NIOSH)	III	EPA/600/R-16/114	III
Disulfoton sulfoxide oxon	2496-92-6	GC-MS / GC-FPD	EPA/600/R-16/114	III	525.2[7,10] (EPA OW)	III	525.2[7,10] (EPA OW)	III	5600 (NIOSH)	III	EPA/600/R-16/114	III
1,4-Dithiane (degradation product of HD)	505-29-3	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	II	EPA/600/R-16/114	II	Not of concern**	NA	EPA/600/R-16/114	II

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
EA 2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid] (hydrolysis product of VX)	73207-98-4	HPLC-UV / LC-MS-MS	3541/3545A (EPA SW-846) EPA/600/R-15/097	*III	EPA/600/R-15/097	*II	EPA/600/R-15/097	*II	TO-10A (EPA ORD)	*III	3570/8290A Appendix A (EPA SW-846) EPA/600/R-15/097	*III
Ethyl methylphosphonic acid (EMPA) (degradation product of VX)	1832-53-7	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224	II
Ethyldichloroarsine (ED)	598-14-1	GC-MS	3541/3545A (EPA SW-846) 8270E (EPA SW-846)	III	3535A (EPA SW-846) 8270E (EPA SW-846)	III	3535A (EPA SW-846) 8270E (EPA SW-846)	III	TO-15 (EPA ORD)	III	9102 (NIOSH) 8270E (EPA SW-846)	III
N-Ethyldiethanolamine (EDEA) (degradation product of HN-1)	139-87-7	LC-MS-MS / IC- conductivity detection	3541/3545A (EPA SW-846) EPA/600/R-11/143	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	III	EPA/600/R-11/143	II
Ethylene oxide	75-21-8	GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	TO-15 (EPA ORD)	I	Not of concern**	NA
Fenamiphos	22224-92-6	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	II	525.2[7] (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Fentanyl	437-38-7	LC-MS-MS	3541/3545A (EPA SW-846) Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649	III	Not of concern**	NA	PHILIS SOP L-A-309 Rev. 0 / L-A-310 Rev. 1	II
Fluoride	16984-48-8	IC-conductivity detection	Not of concern**	NA	300.1, Rev 1.0 (EPA OW)	I	300.1, Rev 1.0 (EPA OW)	I	Not of concern**	NA	Not of concern**	NA
Fluoroacetamide	640-19-7	GC-MS	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA	LC-MS / LC-MS-MS	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III	EPA/600/R-18/056	II	EPA/600/R-18/056	II	S301-1 (NIOSH) Adapted from J. Chromatogr. A (2007) 1139: 271-278	III	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III
2-Fluoroethanol	371-62-0	GC-MS / GC-FID	5035A (EPA SW-846) 8260D (EPA SW-846)	III	5030C (EPA SW-846) 8260D (EPA SW-846)	III	5030C (EPA SW-846) 8260D (EPA SW-846)	III	2513 (NIOSH)	III	Not of concern**	NA

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Fluorosilicic acid (analyze as fluoride)	16961-83-4	IC-conductivity detection	Not of concern**	NA	300.1, Rev 1.0 (EPA OW)	I	300.1, Rev 1.0 (EPA OW)	I	Not of concern**	NA	Not of concern**	NA
Formaldehyde	50-00-0	FGC-ECD / HPLC-UV	8315A (EPA SW-846)	I	8315A (EPA SW-846)	I	556.1 (EPA OW)	I	2016 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8315A (EPA SW-846)	III
Gasoline range organics	NA	GC-FID	5035A (EPA SW-846) 8015D (EPA SW-846)	I	5030C (EPA SW-846) 8015D (EPA SW-846)	I	5030C (EPA SW-846) 8015D (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8015D (EPA SW-846)	I
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	HPLC-UV	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	I
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	LC-MS	8330B (EPA SW-846) Adapted from Analyst (2001) 126:1689-1693	II	3535A/8330B (EPA SW-846) Adapted from Analyst (2001) 126:1689-1693	II	3535A/8330B (EPA SW-846) Adapted from Analyst (2001) 126:1689-1693	II	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) Adapted from Analyst (2001) 126:1689-1693	III
Hydrogen bromide	10035-10-6	IC-conductivity detection	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	7907 (NIOSH)	I	Not of concern**	NA
Hydrogen chloride	7647-01-0	IC-conductivity detection	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	7907 (NIOSH)	I	Not of concern**	NA
Hydrogen cyanide	74-90-8	Visible spectrophotometry	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	6010 (NIOSH)	I	Not of concern**	NA
Hydrogen fluoride	7664-39-3	IC-conductivity detection	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	7906 (NIOSH)	I	Not of concern**	NA
Hydrogen sulfide	7783-06-4	IC-conductivity detection	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	6013 (NIOSH)	I	Not of concern**	NA
Isopropyl methylphosphonic acid (IMPA) (degradation product of GB)	1832-54-8	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224	II

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Kerosene	64742-81-0	GC-FID	3541/3545A (EPA SW-846) 8015D (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8015D (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8015D (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8015D (EPA SW-846)	I
Lead arsenate (analyze as total arsenic)	7645-25-2	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3	ICP-AES / ICP-MS / LC-MS-MS	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	EPA/600/R-15/258[5]	*II	IO-3.1[6] (EPA ORD) IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*II
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8	ICP-AES / ICP-MS / LC-MS-MS	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	IO-3.1[6] (EPA ORD) IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*III
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1	ICP-AES / ICP-MS / LC-MS-MS	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	IO-3.1[6] (EPA ORD) IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*III
Lewisite oxide (degradation product of Lewisite)	1306-02-1	ICP-AES / ICP-MS / LC-MS-MS	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	EPA/600/R-15/258[5]	*III	IO-3.1[6] (EPA ORD) IO-3.4/IO-3.5[6] (EPA ORD)	*I	EPA/600/R-15/258[5]	*III
Mercuric chloride (analyze as total mercury)	7487-94-7	Visible spectrophotometry / CVAA / CVAFS	7473[11] (EPA SW-846)	I	245.1[12] (EPA OW)	I	245.1 (EPA OW)	I	Not of concern**	NA	9102 (NIOSH) 7473[11] (EPA SW-846)	I
Mercury, Total	7439-97-6	Visible spectrophotometry / CVAA / CVAFS	7473[11] (EPA SW-846)	I	245.1[12] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH) 7473[11] (EPA SW-846)	I
Methamidophos	10265-92-6	LC-MS-MS	J.Env.Sci. Health (2014) 49: 23-34 538 (EPA OW)	II	538 (EPA OW)	I	538 (EPA OW)	I	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III
Methomyl	16752-77-5	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	II	531.2 (EPA OW)	I	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	Visible spectrophotometry / CVAA / CVAFS	7473[11] (EPA SW-846)	I	245.1[12] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH) 7473[11] (EPA SW-846)	I
Methyl acrylonitrile	126-98-7	HPLC-UV / GC-MS	5035A (EPA SW-846) 8260D (EPA SW-846)	II	524.2[1] (EPA OW)	II	524.2[1] (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846) 8260D (EPA SW-846)	III

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Methylamine	74-89-5	HPLC-FL / HPLC-vis	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	OSHA 40	I	Not of concern**	NA
N-Methyldiethanolamine (MDEA) (degradation product of HN-2)	105-59-9	LC-MS-MS / IC-conductivity detection	3541/3545A (EPA SW-846) EPA/600/R-11/143	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	III	EPA/600/R-11/143	II
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	GC-MS	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	EPA/600/R-16/115	*III	TO-17[4] (EPA ORD)	*III	EPA/600/R-16/115	*III
3-Methyl fentanyl	42045-87-4	LC-MS-MS	3541/3545A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	3520C/3535A (EPA SW-846) Adapted from J. Chromatogr. B (2014) 962: 52-58	III	Not of concern**	NA	PHILIS SOP L-A-309 Rev. 0 / L-A-310 Rev. 1	III
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	LC-MS	J. Chromatogr. A (2007) 1139: 271-278	III	EPA/600/R-18/056	II	EPA/600/R-18/056	II	S301-1 (NIOSH) J. Chromatogr. A (2007) 1139: 271-278	III	J. Chromatogr. A (2007) 1139: 271-278	III
Methyl hydrazine	60-34-4	Visible spectrophotometry/ HPLC-UV	3541/3545A (EPA SW-846) J. Chromatogr. B (1993) 617: 157-162	III	J. Chromatogr. B (1993) 617: 157-162	III	J. Chromatogr. B (1993) 617: 157-162	III	3510 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) J. Chromatogr. B (1993) 617: 157-162	III
Methyl isocyanate	624-83-9	HPLC-UV	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	OSHA 54	I	Not of concern**	NA
Methyl paraoxon	950-35-6	GC-MS	EPA/600/R-16/114	III	3535A (EPA SW-846) 8270E[10] (EPA SW-846)	III	3535A (EPA SW-846) 8270E[10] (EPA SW-846)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Methyl parathion	298-00-0	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	I	3535A (EPA SW-846) 8270E (EPA SW-846)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114	II
Methylphosphonic acid (MPA) (degradation product of VX, GB, & GD)	993-13-5	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224	II
Mevinphos	7786-34-7	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	I	525.2[7] (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Monocrotophos	6923-22-4	GC-MS	3541/3545A (EPA SW-846) 8270E (EPA SW-846)	I	3535A (EPA SW-846) 8270E (EPA SW-846)	I	3535A (EPA SW-846) 8270E (EPA SW-846)	I	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846) 8270E (EPA SW-846)	III
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8	GC-MS	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653	*II
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2	GC-MS	EPA/600/R-12/653	*III	EPA/600/R-12/653	*III	EPA/600/R-12/653	*III	TO-17 (EPA ORD)	*III	EPA/600/R-12/653	*III
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1	GC-MS	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653	*II
Mustard, sulfur / Mustard gas (HD)	505-60-2	GC-MS	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	TO-17 (EPA ORD)	*II	EPA/600/R-16/115	*I
Nicotine compounds (analyze as nicotine)	54-11-5	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	II	3535A (EPA SW-846) 8270E (EPA SW-846)	II	Not of concern**	NA	EPA/600/R-16/114	II
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	HPLC-UV	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	I
Osmium tetroxide (analyze as total osmium)	20816-12-0	ICP-AES / ICP-MS	3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	II	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	II	3015A (EPA SW-846) 6010D/6020B (SW-846)	II	IO-3.1 (EPA ORD) IO-3.4 (EPA ORD)	II	3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	III
Oxamyl	23135-22-0	HPLC-UV / HPLC-FL / LC-MS-MS	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Paraoxon	311-45-5	GC-MS	EPA/600/R-16/114	III	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	III	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Paraquat	4685-14-7	HPLC-UV / LC-MS-MS	Adapted from J. Chromatogr. A (2008) 1196-1197:110-116	II	549.2 (EPA OW)	I	549.2 (EPA OW)	I	Not of concern**	NA	Adapted from J. Chromatogr. A (2008) 1196-1197:110-116	III
Parathion	56-38-2	GC-MS	EPA/600/R-16/114	II	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	I	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114	II

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Pentaerythritol tetranitrate (PETN)	78-11-5	HPLC-UV	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	I
Phencyclidine	77-10-1	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	II	EPA/600/R-16/114	II	TO-10A (EPA ORD)	II	9106/9109 (NIOSH)	II
Phorate	298-02-2	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E[10] (EPA SW-846)	I	3535A (EPA SW-846) 8270E[10] (EPA SW-846)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Phorate sulfone	2588-04-7	GC-MS / LC-MS-MS	EPA/600/R-16/114	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Phorate sulfone oxon	2588-06-9	GC-MS / LC-MS-MS	EPA/600/R-16/114	III	540 (EPA OW)	III	540 (EPA OW)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Phorate sulfoxide	2588-03-6	GC-MS / LC-MS-MS	EPA/600/R-16/114	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Phorate sulfoxide oxon	2588-05-8	GC-MS / LC-MS-MS	EPA/600/R-16/114	III	540 (EPA OW)	III	540 (EPA OW)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114	III
Phosgene	75-44-5	GC-NPD	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	OSHA 61	I	Not of concern**	NA
Phosphamidon	13171-21-6	GC-MS	EPA/600/R-16/114	II	3520C/3535A (EPA SW-846) 8270E (EPA SW-846)	I	525.3 (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Phosphine	7803-51-2	Visible spectrophotometry	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	6002 (NIOSH)	I	Not of concern**	NA
Phosphorus trichloride	7719-12-2	Visible spectrophotometry	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	6402 (NIOSH)	I	Not of concern**	NA
Pinacolyl methyl phosphonic acid (PMPA) (degradation product of GD)	616-52-4	HPLC-UV / LC-MS-MS	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224	II

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Propylene oxide	75-56-9	GC-MS / GC-FID	5035A (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	5030C (EPA SW-846) 8260D (EPA SW-846)	II	1612 (NIOSH)	I	Not of concern**	NA
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	GC-MS	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653	*II
Sarin (GB)	107-44-8	GC-MS	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	TO-17[4] (EPA ORD)	*II	EPA/600/R-16/115	*I
Sodium arsenite (analyze as total arsenic)	7784-46-5	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Sodium azide (analyze as azide ion)	26628-22-8	IC-conductivity detection	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[13] 300.1, Rev 1.0[14] (EPA OW)	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[13] 300.1, Rev 1.0[14] (EPA OW)	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[13] 300.1, Rev 1.0[14] (EPA OW)	II	ID-211 (OSHA)	I	ID-211 (OSHA)	I
Soman (GD)	96-64-0	GC-MS	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	EPA/600/R-16/115	*I	TO-17[4] (EPA ORD)	*II	EPA/600/R-16/115	*I
Strychnine	57-24-9	GC-MS	EPA/600/R-16/114	II	3535A (EPA SW-846) 8270E (EPA SW-846)	I	3535A (EPA SW-846) 8270E (EPA SW-846)	I	Not of concern**	NA	EPA/600/R-16/114	II
Tabun (GA)	77-81-6	GC-MS	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	EPA/600/R-12/653	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653	*II
Tetraethyl pyrophosphate (TEPP)	107-49-3	GC-MS	EPA/600/R-16/114	II	3511 (EPA SW-846) 8270E (EPA SW-846)	II	3511 (EPA SW-846) 8270E (EPA SW-846)	II	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Tetramethylenedisulfotetramine (TETS)	80-12-6	GC-MS	EPA/600/R-16/114	II	EPA/600/R-16/114	I	EPA/600/R-16/114	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114	II
Thallium sulfate (analyze as total thallium)	10031-59-1	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
Thiodiglycol (TDG) (degradation product of HD)	111-48-8	HPLC-UV / LC-MS-MS	E2787-11 (ASTM)	II	D7598-16 (ASTM)	II	D7598-16 (ASTM)	III	TO-10A (EPA ORD)	III	E2838-11 (ASTM)	II
Thiofanox	39196-18-4	HPLC-UV / LC-MS-MS	3541/3545A (EPA SW-846) D7645-16 (ASTM)	III	D7645-16 (ASTM)	II	538 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846) D7645-16 (ASTM)	III

Analyte(s)	CAS RN	Determinative Technique	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
1,4-Thioxane (degradation product of HD)	15980-15-1	GC-MS	EPA/600/R-16/114[15]	II	EPA/600/R-16/114[15]	II	EPA/600/R-16/114[15]	II	Not of concern**	NA	EPA/600/R-16/114[15]	II
Titanium tetrachloride (analyze as total titanium)	7550-45-0	ICP-AES / ICP-MS	3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	Not of concern**	NA	Not of concern**	NA	Not of concern**	NA	3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	III
Triethanolamine (TEA) (degradation product of HN-3)	102-71-6	LC-MS-MS / IC-conductivity detection	3541/3545A (EPA SW-846) EPA/600/R-11/143	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	II	EPA/600/R-11/143	II
Trimethyl phosphite	121-45-9	GC-MS	3541/3545A (EPA SW-846) 8270E[16] (EPA SW-846)	III	Not of concern**	NA	Not of concern**	NA	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846) 8270E[16] (EPA SW-846)	III
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4	HPLC-UV	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	I
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7	HPLC-UV	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846) 8330B (EPA SW-846)	I	Not of concern**	NA	3570/8290A Appendix A (EPA SW-846) 8330B (EPA SW-846)	I
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	ICP-AES / ICP-MS	3050B/3051A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	3015A (EPA SW-846) 6010D/6020B (EPA SW-846)	I	200.7/200.8[3] (EPA OW)	I	IO-3.1 (EPA ORD) IO-3.4/IO-3.5 (EPA ORD)	I	9102 (NIOSH) 6010D/6020B (EPA SW-846)	I
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0	GC-MS	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116	*III
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5	GC-MS	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116	*III
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5	GC-MS	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	EPA/600/R-16/116	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116	*III
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9	GC-MS	EPA/600/R-16/116	*II	EPA/600/R-16/116	*I	EPA/600/R-16/116	*I	TO-17 (EPA ORD)	*II	EPA/600/R-16/116	*II
White phosphorus	12185-10-3	GC-NPD / GC-FPD	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7905 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 7580 (EPA SW-846)	III

* Only laboratories approved under the ERLN umbrella are designated for handling the CWA standards needed for this method. For access to the nearest ERLN laboratory specially trained and equipped for CWA analysis, contact the EPA Headquarters Emergency Operations Center (EPA/HQ-EOC) at 202-564-3850.

** In some cases, analytes are listed as not a concern in a particular sample type; in these cases, SAM work groups have determined that the analyte is not a concern due to a number of factors, including the analyte's low likelihood of persistence, toxicity, mobility or solubility within the particular sample type.

Footnotes

[1] Methods 524.3 or 524.4 may be used in place of Method 524.2 provided the laboratory has the necessary equipment and expertise.

[2] If problems occur when using this method, TO-10A should be used.

[3] Laboratories with demonstrated expertise in collision/reaction cell procedures have the option of using SW-846 Method 3015A (sample preparation) and Method 6020B (determination).

[4] If problems occur when using this method, Method TO-15 should be used.

[5] The following methods can be used to analyze these compounds as total arsenic in situations where high throughput analysis is needed or where standards are not available for the specific compounds: ICP AES/MS Methods 200.7/200.8 for drinking water; Methods 3015A/6010D/6020B for non-drinking water samples; Methods 3050B/3051A/6010D/6020B for solid samples; and Methods 9102/6010D/6020B for wipes.

[6] TO Methods IO-3.1, IO-3.4 and IO-3.5 address these compounds as total arsenic in air samples.

[7] Method 525.3 may be used in place of Method 525.2 provided the laboratory has the necessary equipment and expertise.

[8] Standard Method 4500-CN-G may be used in place of RLAB Method 3135.2I for the analysis of cyanide amenable to chlorination in non-drinking water or drinking water samples.

[9] The inline distillation method, EPA-821-B-01-009, may be used to prepare and analyze for total cyanide in non-drinking water samples.

[10] If problems occur during measurement of oxon compounds, analysts should consider use of procedures included in Kamal, A. et al. "Oxidation of selected organophosphate pesticides during chlorination of simulated drinking water." Water Research. 2009. 43(2): 522-534. https://www.sciencedirect.com/science/article/abs/pii/S0043135408004995.

[11] If equipment is not available or problems occur when analyzing solid and wipe samples, use CVAA Method 7471B (EPA SW-846).

[12] If problems occur when using EPA Method 245.1 for these analytes during preparation and analysis of non-drinking water samples, refer to EPA Method 7470A (SW-846).

[13] Water extraction, filtration and acidification steps from the Journal of Forensic Science. 1998. 43(1): 200-202 should be used for the preparation of solid samples. Filtration and acidification steps from this journal should be used for preparation of non-drinking water and drinking water samples.

[14] If analyses are problematic, refer to column manufacturer for alternate conditions.

[15] If problems occur when using this method, SW-846 Method 8260D and appropriate corresponding sample preparation procedures (i.e., 5035A for solid samples, and 5030C for aqueous liquid and drinking water samples) should be used.

[16] If problems occur with analyses, lower the injection temperature.

Appendix B1: Selected Radiochemical Methods for Environmental Samples

SAM 2022 — Appendix B1: Selected Radiochemical Methods for Environmental Samples

***Note*: Column headings are defined in Section 6.0.**

Analyte Class	Determinative Technique	Drinking Water	Aqueous and Liquid Phase	Soil and Sediment	Surface Wipes	Air Filters	Vegetation
Gross Alpha	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gross Beta	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gamma	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Select Mixed Fission Products[1]	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Total Activity Screening	Liquid scintillation	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Actinium-225[3]	14265-85-1	Alpha counting / Alpha spectrometry / Gamma spectrometry	900.0 (EPA)	Determination of Actinium-225 in Water Samples (Eichrom)	7110 B (SM)	Determination of Actinium-225 in Water Samples (Eichrom)	AP1 (ORISE)	Determination of Actinium-225 in Geological Samples (Eichrom)	FRMAC, Vol 2, pg. 33 (DOE)	Determination of Actinium-225 in Geological Samples (Eichrom)	FRMAC, Vol 2, pg. 33 (DOE)	Determination of Actinium-225 in Geological Samples (Eichrom)	AP1 (ORISE)	Determination of Actinium-225 in Geological Samples (Eichrom)
Americium-241[4]	14596-10-2	Alpha spectrometry	Rapid Radiochemical Method for Americium-241[5] (EPA)	Am-04-RC (HASL-300)	D3084-20 (ASTM)	Am-04-RC (HASL-300)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	Am-01-RC[6] (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
		Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Californium-252[4]	13981-17-4	Alpha spectrometry	D3084-20 (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-20 (ASTM)	Am-06-RC (HASL-300)
Cesium-137	10045-97-3	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Cobalt-60	10198-40-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Curium-244[4]	13981-15-2	Alpha spectrometry	D3084-20 (ASTM)	Rapid Radiochemical Method for Curium-244 in Water (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Curium-244 in Water (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-20 (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-20 (ASTM)	Am-06-RC (HASL-300)
Europium-154	15585-10-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Gallium-68[7]	15757-14-9	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Germanium-68[7]	15756-77-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Indium-111	15750-15-9	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iodine-125	14158-31-7	Gamma spectrometry	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9[8] (ORISE)	Procedure #9[8] (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)
Iodine-131	10043-66-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R[8] (HASL-300)	Ga-01-R[8] (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iridium-192	14694-69-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Molybdenum-99	14119-15-4	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Neptunium-237	13994-20-2	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)
Neptunium-239	13968-59-7	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Phosphorus-32	14596-37-3	Liquid scintillation / Beta counting	Rapid Radiochemical Method for Phosphorous-32 in water[5] (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)
Plutonium-238[4]	13981-16-3	Alpha spectrometry	Rapid Radiochemical Method for Plutonium-238 and -239/240[5] (EPA)	EMSL-33 (EPA)	D3084-20 (ASTM)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Plutonium-239[4]	15117-48-3	Alpha spectrometry	Rapid Radiochemical Method for Plutonium-238 and 239/240[5] (EPA)	EMSL-33 (EPA)	D3084-20 (ASTM)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Polonium-210	13981-52-7	Alpha spectrometry	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Radium-223	15623-45-7	Alpha spectrometry	Rapid Radiochemical Method for Radium-226[5] (EPA)		Rapid Radiochemical Method for Radium-226 (EPA)		Rapid Radiochemical Method for Radium-226 (EPA)		Rapid Radiochemical Method for Radium-226 (EPA)		Rapid Radiochemical Method for Radium-226 (EPA)		Rapid Radiochemical Method for Radium-226 (EPA)	
Radium-226	13982-63-3	Alpha spectrometry / Alpha counting / Gamma spectrometry	Rapid Radiochemical Method for Radium-226[5] (EPA)	Method for Radium-228 and Radium-226 in Drinking Water (GA Tech)	7500-Ra B (SM)	7500-Ra C (SM)	Rapid Method for Radium in Soil (EPA)	AP7 (ORISE)	Rapid methods* for acid or fusion digestion (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid methods* for acid or fusion digestion (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Ra-03-RC (HASL-300)	Ra-03-RC (HASL-300)
Rhenium-188	14378-26-8	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Rubidium-82[9]	14391-63-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Ruthenium-103	13968-53-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Ruthenium-106	13967-48-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Selenium-75	14265-71-5	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Strontium-89	14158-27-1	Beta counting	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)	Strontium in Food and Bioenvironmental Samples (EPA)		Strontium in Food and Bioenvironmental Samples (EPA)		Actinides and Sr-89/90 in Vegetation (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)
Strontium-90	10098-97-2	Beta counting / Gamma spectrometry	Rapid Radiochemical Method for Radiostrontium[5] (EPA)	905.0 (EPA)	D5811-20 (ASTM)	D5811-20 (ASTM)	Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices (EPA)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Sr-03-RC (HASL-300)
Technetium-99	14133-76-7	Liquid scintillation / Beta counting / Gamma spectrometry	Tc-02-RC (HASL-300)	Tc-02-RC (HASL-300)	D7168-16 (ASTM)	D7168-16 (ASTM)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	Tc-01-RC (HASL-300)
Technetium-99m	378784-45-3	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Thorium-227	15623-47-9	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Thorium-228	14274-82-9	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Thorium-230	14269-63-7	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Thorium-232	7440-29-1	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Tritium (Hydrogen-3)	10028-17-8	Liquid scintillation	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	Not applicable[10]	Not applicable[10]	AP2 (ORISE)	AP2 (ORISE)
Uranium-234[4]	13966-29-5	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[5] (EPA)	D3972-09 (2015) (ASTM)	7500-U B[11] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)
Uranium-235[4]	15117-96-1	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[5] (EPA)	D3972-09 (2015) (ASTM)	7500-U B[11] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)
Uranium-238[4]	7440-61-1	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[5] (EPA)	D3972-09 (2015) (ASTM)	7500-U B[11] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)

* These rapid methods describe wipe and air filter digestion procedures, and include references to the analyte-specific separation procedures listed for rapid analysis of drinking water samples, to be used to complete analysis of the digested samples.

Footnotes

[1] Please note that this category does not cover all fission products. In addition to the specific radionuclides listed in this appendix, gamma-ray spectrometry with a high resolution HP(Ge) detector will identify and quantify fission products with gamma rays in the energy range of 30 keV to 2000 keV. The sensitivity will be dependent on the detector efficiency and the gamma-ray emission probabilities (branching ratio) for the specific radionuclide.

[2] In those cases where the same method is listed for qualitative determination and confirmatory analysis, qualitative determination can be performed by application of the method over a shorter count time than that used for confirmatory analysis.

[3] Gross alpha screening may be used for qualitative analysis of actinium-225. For every one actinium-225 decay, there are up to four alpha particles emitted depending on daughter equilibrium. To determine the qualitative result for actinium-225, the gross alpha result should be divided by four.

[4] If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 for qualitative determination or confirmatory analysis of alpha radioactivity.

[5] This method is listed for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

[6] In cases where only small sample volumes (≤100 g) are available, use HASL-300 Method Pu-12-RC.

[7] For qualitative analysis of the germanium-68 and gallium-68 pair, long count times may be required to meet detection limits as the 1077 KeV peak has a 3% abundance; for confirmatory analysis, the 511 KeV (176% abundance) should be larger than normal.

[8] This procedure should be used only for filters specifically designed for iodine.

[9] When detecting rubidium-82 (75 second half-life) by gamma spectroscopy in environmental samples, it is measured in equilibrium with its parent, strontium-82 (25.5 day half-life).

[10] Because tritium is not sampled using traditional air filters, this matrix is not applicable.

[11] This method was developed for measurement of total uranium and does not distinguish between uranium isotopes.

Appendix B2: Selected Rapid Radiochemical Methods for Outdoor Building and Infrastructure Materials

SAM 2022 — Appendix B2: Selected Rapid Radiochemical Methods for Outdoor Building and Infrastructure Materials

***Note*: Column headings are defined in Section 6.0.**

Analyte(s)	CAS RN	Determinative Technique	Asphalt Shingles		Asphalt Matrices (Paving Materials)		Concrete		Brick		Limestone	
			Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis
Americium-241	14596-10-2	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)
Plutonium-238	13981-16-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)
Plutonium-239	15117-48-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Plutonium-238 and Plutonium-239/240 in Building Materials (EPA)
Radium-226	13982-63-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)
Strontium-90	10098-97-2	Beta counting	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)
Uranium-234	13966-29-5	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)
Uranium-235	15117-96-1	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)
Uranium-238	7440-61-1	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Limestone Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)

Appendix C: Selected Pathogen Methods

SAM 2022 — Appendix C: Selected Pathogen Methods

Not all methods have been evaluated for each pathogen/sample type/environmental matrix combination in Appendix C. Each laboratory using these methods must operate a formal quality assurance program and, at a minimum, analyze appropriate quality control (QC) samples (Section 7.1.2). Also, if required, a modification or an appropriate replacement method may be warranted for a specific pathogen/sample type/environmental matrix or a combination thereof. Additionally, the SAM Pathogen primary and alternate points of contact should be consulted for additional guidance (Section 4.0, Points of Contact).

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected pathogen methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers pertain only to technical aspects of method usability, and do not pertain to aspects such as cost, equipment availability, and sample throughput. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The method was developed for the pathogen and sample type. The method has been evaluated by multiple laboratories, a detailed protocol has been developed, and suitable QC measures and checks are provided. (Examples: EPA Method 1623.1 [*Cryptosporidium* in water]; Standard Methods 9260 E [*Shigella* culture method].)

Tier II: The pathogen is the target of the method, and the method has been evaluated by one or more laboratories. The available data and/or information indicate that additional testing and/or modifications will likely be needed. (Example: Cunningham et al. 2010. [*Shigella* molecular method].)

Tier III: The pathogen is not the target of the method but the method is for the specific sample type and the pathogen is similar to the target of the method (i.e. vegetative bacteria, spore-forming bacteria, virus or protozoan). Data and expert opinion suggest, however, that the method(s) may be applicable with modifications. (Example: EPA *Yersinia pestis* protocol for *Chlamydophila psittaci* in water.)

***Notes*:**
Samples should not be stored indefinitely, and should be processed and analyzed as soon as possible upon receipt.

If viability determinations are needed (e.g., for post decontamination phase samples), a viability-based procedure (such as culture) should be used. Rapid analysis techniques (such as PCR, immunoassays) without culture are preferred for determination of the extent and magnitude of contamination (e.g., for site characterization phase samples). Please see Figure 7-1.

Column headings are defined in Section 7.0.

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Bacteria[2]										
Bacillus anthracis [Anthrax]	NA	Sample Processing	EPA *Bacillus anthracis* (BA) Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	Silvestri et al. 2016. J. of Microbiol. Methods. 130: 6-13	II	EPA BA Protocol (EPA/600/R-17/213)	I
	Culture	Analytical Technique	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I
	Real-time PCR/ RV-PCR									
Brucella spp. (*B. abortus, B. melitensis, B. suis*) [Brucellosis]	NA	Sample Processing	EPA *Yersinia pestis* (YP) Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I
	Real-time PCR	Analytical Technique	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Burkholderia mallei [Glanders] and *Burkholderia pseudomallei* [Melioidosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Hall et al. 2019. PLoS Negl. Trop. Dis. 13(9):e0007727	II	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I
	Real-time PCR	Analytical Technique	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II
Campylobacter jejuni [Campylobacteriosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Hiett. 2017. Methods Mol. Biol. 1512:1-8	II	Hiett. 2017. Methods Mol. Biol. 1512:1-8	II
	Culture	Analytical Technique	ISO 17995	I	ISO 17995	I	ISO 17995	I	ISO 17995	I
	Real-time PCR	Analytical Technique	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*) [Psittacosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Tissue culture PCR	Analytical Technique	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II
Coxiella burnetii [Q-fever]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA and CDC Joint Collection Protocol (Ultrafiltration [UF]) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II
	Real-time PCR	Analytical Technique	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Escherichia coli O157:H7	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1680 (EPA-821-R-14-009)	I	EPA *Eschericia coli* O157:H7 (EC) Protocol (EPA/600/R-10/056)	I
	Culture	Analytical Technique	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I
	Real-time PCR	Analytical Technique	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II
Francisella tularensis [Tularemia]	NA	Sample Processing	EPA *Francisella tularensis* (FT) Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA Method 1682 (EPA-821-R-06-14)	III	EPA FT Protocol (EPA/600/R-19/110)	I
	Culture	Analytical Technique	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I
	Real-time PCR/ RV-PCR	Analytical Technique	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I
Legionella pneumophila [Legionellosis]	NA	Sample Processing	US DHHS. 2005. Procedures for the Recovery of *Legionella* from the Environment	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I
	Culture	Analytical Technique	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I
	Real-time PCR	Analytical Technique	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I
Leptospira interrogans [Leptospirosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Standard Method 9260 I: *Leptospira*	I
	Culture	Analytical Technique	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I
	Real-time PCR	Analytical Technique	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II
Listeria monocytogenes [Listeriosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Iwu and Okoh. 2020. PLoS ONE. 15(2): e0228956.	II	Iwu and Okoh. 2020. PLoS ONE. 15(2): e0228956.	II
	Culture	Analytical Technique	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I
	Real-time PCR	Analytical Technique	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	I	EPA Method 1200 (EPA 817-R-12-004)	I
	Culture	Analytical Technique	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I
	Real-time PCR	Analytical Technique	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II
Salmonella Typhi [Typhoid fever]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	I	EPA *Salmonella* Typhi (ST) Protocol (EPA 600/R-10/133)	I
	Culture	Analytical Technique	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I
	Real-time PCR	Analytical Technique	CDC Laboratory Assay	I	CDC Laboratory Assay	I	CDC Laboratory Assay	I	CDC Laboratory Assay	I
Shigella spp. [Shigellosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Standard Method 9260 E: *Shigella*	I
	Culture	Analytical Technique	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I
	Real-time PCR	Analytical Technique	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II
Staphylococcus aureus	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Li et al. 2015. Environ. Sci. Technol. 49: 14249-14256	II
	Culture	Analytical Technique	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I
	Real-time PCR	Analytical Technique	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II
Vibrio cholerae [Cholera]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA *Vibrio cholerae* (VC) Protocol (EPA 600/R-10/139)	I
	Culture	Analytical Technique	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I
	Real-time PCR	Analytical Technique	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Yersinia pestis [Plague]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	I
	Culture Real-time PCR/ RV-PCR	Analytical Technique	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I
Viruses[3]										
Adenoviruses: Enteric and non-enteric (A-F)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977.	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67.	II	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II
	Real-time PCR	Analytical Technique	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II
Astroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Integrated Cell Culture Real-time reverse transcription-PCR	Analytical Technique	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Caliciviruses: Noroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Real-time reverse transcription-PCR	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I
Caliciviruses: Sapovirus	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II
	Real-time reverse transcription-PCR	Analytical Technique	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II
Coronaviruses: SARS-associated human coronavirus (SARS-CoV-2, SARS-CoV, and MERS-CoV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II
	Real-time reverse transcription-PCR	Analytical Technique	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II
	Rapid viability-reverse transcription-PCR	Analytical Technique	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Hepatitis E virus (HEV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II
Influenza H5N1 virus	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	II	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67.	III	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II
	Real-time reverse transcription-PCR	Analytical Technique	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II
Picornaviruses: Enteroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I
	Reverse transcription-PCR									

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Picornaviruses: Hepatitis A virus (HAV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Integrated Cell Culture	Analytical Technique	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II
	Real-time Reverse Transcription-PCR									
Reoviruses: Rotavirus (Group A)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Protozoa										
Cryptosporidium spp. [Cryptosporidiosis]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Zopp et al. 2016. Agric. Environ. Lett. 1:160031	II	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	I/I/III
	Cell Culture Immunofluorescence Procedure	Analytical Technique	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II
	IMS/FA	Analytical Technique	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II
Entamoeba histolytica	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Ogbolu et al. 2011. Afr. J. Med. med. Sci. 40: 85-87	II	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Cell Culture	Analytical Technique	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II
	Real-time PCR	Analytical Technique	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Giardia spp. [Giardiasis]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	III	EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	I/III
	Cell Culture	Analytical Technique	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II
	IMS/FA	Analytical Technique	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II
Naegleria fowleri [Naegleriasis]	NA	Sample Processing	Not of concern[4]		Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Cope et al. 2015. Clin. Infect. Dis. 60(8): e36-42 or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	II/III
	Cell Culture	Analytical Technique	Not of concern[4]		Standard Method 9750: *Naegleria fowleri*	I	Standard Method 9750: *Naegleria fowleri*	I	Standard Method 9750: *Naegleria fowleri*	I
	Real-time PCR	Analytical Technique	Not of concern[4]		Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II
Toxoplasma gondii [Toxoplasmosis]	NA	Sample Processing	Lass et al. 2020. Parasitol. 1-11	II	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Escotte-Binet et al. 2019. Vet. Parasitol. 274: 108904	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225 or EPA Method 1623.1 (EPA 816-R-12-001)	II/III
	Cell Culture	Analytical Technique	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II
	Real-time PCR	Analytical Technique	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Helminths										
Baylisascaris procyonis [Raccoon roundworm infection]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Kazacos. 1983. AM. J. Vet. Res. Vol 44. No. 5: 896-900	II	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001) or Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	III/II
	Real-time PCR	Analytical Technique	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II
	Embryonation of Eggs and Microscopy	Analytical Technique	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II

Footnotes

[1] A neutralizing agent (e.g., sodium thiosulfate) should be added to water samples that may have disinfectant residuals prior to sample processing and analysis. Additional sample processing may be required for wastewater samples to remove solids (see CDC's webpage for additional information on processing wastewater samples for viruses: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html).

[2] If the water sample processing method for bacterial analyses does not address large volume water samples, please refer to the EPA YP Protocol (EPA/600/R-16/109) for ultrafiltration of large volume water samples.

[3] Water samples should be processed according to Method 1642 for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF) and Method 1642 (filter processing) for volumes ≥ 10 L.

[4] *Naegleria fowleri* has not been shown to spread via water vapor or aerosol droplets (see CDC's webpage on *Naegleria fowleri* at https://www.cdc.gov/parasites/naegleria/infection-sources.html).

Appendix D: Selected Biotoxin Methods

SAM 2022 — Appendix D: Selected Biotoxin Methods

The fitness of a method for its intended use is related to data quality objectives (DQOs) for a particular environmental remediation activity. The tiers below have been assigned to the methods selected for each biotoxin/sample type pair to indicate a level of method usability for the specific biotoxin and sample type for which it has been selected. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), and appropriate quality control. The sample types indicated reflect respresentative examples and are not necessarily inclusive of all sample types that might be encountered by laboratories following a contamination incident. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The biotoxin and sample type are both targets of the method(s). Data are available for all aspects of method performance and QC measures supporting its use without modifications.

Tier II: The biotoxin is a target of the method, and the method has been evaluated by one or more laboratories. The sample type may or may not be a target of the method, and available data and/or information regarding sample preparation indicate that analyses of similar sample types were successful. However, additional testing and/or modifications may be needed.

Tier III: The sample type is not a target of the method, and no reliable data supporting the method's fitness for its intended use are available. Data suggest, however, that the method(s) may be applicable with significant modification.

***Notes*:**
The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection to slow degradation (e.g., pH adjustors, de-chlorinating agents) could possibly affect analytical results. When present, the impact of these agents on method performance should be evaluated if not previously determined.

Column headings are defined in Section 8.0.

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (air filter, filter cassette, liquid impinger)		Solid (soil, powder)		Particulate (swab, wipe, filter cassette)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Abrin	Abrin (1393-62-0) Abrine (526-31-8)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II
		Presumptive (Abrine)	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
		Presumptive	Immunoassays (ELISA and ECL)	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II
		Confirmatory	LC-MS-MS	Adapted from Analytical Chemistry (2017) 89(21): 11719-11727	II	Adapted from Analytical Chemistry (2017) 89(21): 11719-11727	I	Adapted from Analytical Chemistry (2017) 89(21): 11719-11727	II	Adapted from Analytical Chemistry (2017) 89(21): 11719-11727	I	Adapted from Analytical Chemistry (2017) 89(21): 11719-11727	I
		Biological Activity	Enzyme activity	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II
Aflatoxins	B1 (27261-02-5) B2 (22040-96-6) G1 (1385-95-1) G2 (7241-98-7)	Presumptive (B1, B2, G1, G2)	Immunoaffinity (column) purification / LC-FL (detection)	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II
		Presumptive	Immunoassay (LFA)	See summary in Section 8.2.2.2	III	See summary in Section 8.2.2.2	III	See summary in Section 8.2.2.2	III	See summary in Section 8.2.2.2	III	See summary in Section 8.2.2.2	III
		Presumptive (B1, B2, G1, G2)	Immunoassay (ELISA)	See summary in Section 8.2.2.3	III	See summary in Section 8.2.2.3	III	See summary in Section 8.2.2.3	III	See summary in Section 8.2.2.3	III	See summary in Section 8.2.2.3	III
		Confirmatory (B1, B2, G1, G2)	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (air filter, filter cassette, liquid impinger)		Solid (soil, powder)		Particulate (swab, wipe, filter cassette)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Amanitin	α-amanitin (23109-05-9) β-amanitin (21150-22-1) γ-amanitin (21150-23-2)	Presumptive (α-amanitin)	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Presumptive (α-amanitin β-amanitin γ-amanitin)	Immunoassay (LFA)	Adapted from Toxins (2020) 12(2): 123	II	Adapted from Toxins (2020) 12(2): 123	II	Adapted from Toxins (2020) 12(2): 123	II	Adapted from Toxins (2020) 12(2): 123	II	Adapted from Toxins (2020) 12(2): 123	II
		Confirmatory (α-amanitin)	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
Anatoxin-a	64285-06-9	Presumptive	Immunoassay (ELISA)	Adapted from Inland Waters (2020) 10(1): 109-117	II	Adapted from Inland Waters (2020) 10(1): 109-117	II	Adapted from Inland Waters (2020) 10(1): 109-117	II	Adapted from Inland Waters (2020) 10(1): 109-117	I	Adapted from Inland Waters (2020) 10(1): 109-117	I
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	EPA/600/R-17/130	I	Method 545 (EPA)	I
Botulinum neurotoxins (Serotoypes A, B, C, D, E, F, and G)	Type A (93384-43-1) Type B (93384-44-2) Type C (93384-45-3) Type D (93384-46-4) Type E (93384-47-5) Type F (107231-15-2) Type G (107231-16-3)	Presumptive (Types A and B)	Immunoassay (LFA)	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	I
		Presumptive (Types A, B, D, E, F, and G)	Immunocapture Forster Resonance Energy Transfer (FRET)-based activity assay	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II
		Presumptive (Types A-G)	Immunoassay (fluorescent bead-based)	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II
		Presumptive (Type A)	Immunoassay (ECL)	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II
		Presumptive (Type A)	Immunoassay (B-cell based)	Adapted from Toxins (2018) 10(11): 476	II	Adapted from Toxins (2018) 10(11): 476	II	Adapted from Toxins (2018) 10(11): 476	II	Adapted from Toxins (2018) 10(11): 476	I	Adapted from Toxins (2018) 10(11): 476	I
		Confirmatory (Types A-G)	LC-MS-MS (Types A, B, E and F) MALDI-TOF MS (Types A-G)	Adapted from J. Agric.Food Chem. (2015) 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. (2015) 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. (2015) 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. (2015) 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. (2015) 63(4): 1133-1141	II
		Biological Activity (Total)	Mouse Bioassay	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I
Brevetoxins	98112-41-5 (A-type, congeners BTX-1, BTX-7, BTX-10) 79580-28-2 (B-type, congeners BTX-2, BTX-3, BTX-5, BTX-6, BTX-8, BTX-9)	Presumptive (B-type)	Immunoassay (ELISA)	Adapted from Journal of Shellfish Research (2020) 39(2): 491-500	II	Adapted from Journal of Shellfish Research (2020) 39(2): 491-500	II	Adapted from Journal of Shellfish Research (2020) 39(2): 491-500	II	Adapted from Journal of Shellfish Research (2020) 39(2): 491-500	II	Adapted from Journal of Shellfish Research (2020) 39(2): 491-500	II
		Confirmatory (A and B-types)	LC-MS	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (air filter, filter cassette, liquid impinger)		Solid (soil, powder)		Particulate (swab, wipe, filter cassette)		Non-Drinking Water (surface water, waste water)		Drinking Water	
α-Conotoxins*	Various	Confirmatory	LC-MS	Adapted from Toxins (2017) 9(9): 281	III	Adapted from Toxins (2017) 9(9): 281	III	Adapted from Toxins (2017) 9(9): 281	III	Adapted from Toxins (2017) 9(9): 281	III	Adapted from Toxins (2017) 9(9): 281	III
Cylindrospermopsin	143545-90-8	Presumptive	Immunoassay (ELISA)	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	EPA/600/R-17/130	I	Method 545 (EPA)	I
Deoxynivalenol*	51481-10-8	Confirmatory	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II
Domoic acid (DA)	14277-97-5	Presumptive	Immunoassay (ELISA)	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II
		Presumptive	Immunoassay (LFA)	See summary in Section 8.2.10.3	II	See summary in Section 8.2.10.3	II	See summary in Section 8.2.10.3	II	See summary in Section 8.2.10.3	II	See summary in Section 8.2.10.3	II
		Confirmatory	LC-MS	Adapted from Journal of AOAC International (2014) 97(2): 316-324	II	Adapted from Journal of AOAC International (2014) 97(2): 316-324	II	Adapted from Journal of AOAC International (2014) 97(2): 316-324	II	Adapted from Journal of AOAC International (2014) 97(2): 316-324	II	Adapted from Journal of AOAC International (2014) 97(2): 316-324	II
Fumonisin*	116355-83-0 (B1) 116355-84-1 (B2) 136379-59-4 (B3)	Confirmatory	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II
Microcystins	96180-79-9 (LA) 154037-70-4 (LF) 101043-37-2 (LR) 123304-10-9 (LY) 111755-37-4 (RR) 101064-48-6 (YR)	Presumptive (Total Adda-containing microcystins)	Immunoassay (ELISA)	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	I	Method 546 (EPA)	I
		Confirmatory (Total Adda-containing microcystins)	LC-MS-MS	EPA/600/R-17/344	II	EPA/600/R-17/344	II	EPA/600/R-17/344	II	EPA/600/R-17/344	I	Method 544 (EPA)	I
		Biological Activity (Total Adda-containing microcystins)	Protein phosphatase 2A (PP2A) Activity Assay	Adapted from Toxins (2019) 11(12): 729	II	Adapted from Toxins (2019) 11(12): 729	II	Adapted from Toxins (2019) 11(12): 729	II	Adapted from Toxins (2019) 11(12): 729	II	Adapted from Toxins (2019) 11(12): 729	II
Ochratoxin A*	303-47-9	Confirmatory	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II
Picrotoxin*	124-87-8	Confirmatory	LC-UV	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (air filter, filter cassette, liquid impinger)		Solid (soil, powder)		Particulate (swab, wipe, filter cassette)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Ricin	Ricin (9009-86-3) Ricinine (5254-40-3)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Presumptive	Immunoassay (ECL)	EPA/600/R-22/033A	II	EPA/600/R-22/033A	II	EPA/600/R-22/033A	I	EPA/600/R-22/033A	II	EPA/600/R-22/033A	I
		Presumptive (Ricinine)	LC-MS-MS	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	I
		Presumptive	Time-Resolved Fluorescence (TRF) Immunoassay	CDC LRN**	–	CDC LRN**	–	CDC LRN**	–	CDC LRN**	–	CDC LRN**	–
		Confirmatory	Immunocapture / LC-MS-MS	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	I
		Biological Activity	Immunocapture / MALDI-TOF MS	Adapted from Analytical Chemistry (2016) 88: 6867-6872	II	Adapted from Analytical Chemistry (2016) 88: 6867-6872	II	Adapted from Analytical Chemistry (2016) 88: 6867-6872	II	Adapted from Analytical Chemistry (2016) 88: 6867-6872	II	Adapted from Analytical Chemistry (2016) 88: 6867-6872	I
Saxitoxins	35523-89-8 (STX) 64296-20-4 (NEO) 58911-04-9 (dcSTX) 68683-58-9 (dcNEOSTX) 143084-69-9 (doSTX) 77462-64-7 (GTX 1 - 6) 122075-86-9 (dcGTX 1 - 4)	Presumptive (Total)	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Presumptive (Total)	Immunoassay (ELISA)	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Harmful Algae (2016) 56: 77-90	I	Adapted from Harmful Algae (2016) 56: 77-90	I
		Confirmatory (STXs and GTXs)	LC-MS-MS	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II
Shiga and Shiga-like Toxins	Stx (75757-64-1)	Presumptive (Stx, Stx-1 and Stx-2)	Immunoassay (ELISA)	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	I	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II
		Confirmatory (Stx, Stx-1 and Stx-2)	LC-MS-MS	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II
Staphylococcal enterotoxins	37337-57-8 (SEA) 39424-53-8 (SEB) 39424-54-9 (SEC) 12788-99-7 (SED) 39424-55-0 (SEE)	Presumptive (SEA - SEE)	Enzyme Immunoassay (ELFA)	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II
		Presumptive (SEB)	Immunoassay (ECL)	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III
		Confirmatory (SEA - SEE)	Immunoassay (ELISA)	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (air filter, filter cassette, liquid impinger)		Solid (soil, powder)		Particulate (swab, wipe, filter cassette)		Non-Drinking Water (surface water, waste water)		Drinking Water	
T-2 Mycotoxin	21259-20-1 (T-2) 26934-87-2 (HT-2)	Presumptive (T-2)	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Confirmatory (T-2 and HT-2)	LC-MS	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II
Tetrodotoxin	9014-39-5	Presumptive	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Confirmatory	LC-MS-MS	Adapted from Journal of AOAC International (2017) 100(5): 1469-1482	II	Adapted from Journal of AOAC International (2017) 100(5): 1469-1482	II	Adapted from Journal of AOAC International (2017) 100(5): 1469-1482	II	Adapted from Journal of AOAC International (2017) 100(5): 1469-1482	II	Adapted from Journal of AOAC International (2017) 100(5): 1469-1482	II
Zearalenone*	17924-92-4	Confirmatory	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II	Adapted from Journal of Agricultural and Food Chemistry (2017) 65(33): 7138-7152	II

* At the time of publication, methods for presumptive analysis were not identified. If updates become available, information will be provided on the SAM website: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

**A standardized procedure, reagents and agent-specific algorithms are available only to LRN member laboratories (see Section 7.1.4 of SAM for more information on the LRN).

Attachment 1:
SAM Revisions and Supporting Documents

The information in this document is updated periodically to incorporate revisions to the list of target analytes and sample types, and to provide the most recent analytical methods and procedures. The table below provides information regarding additional changes that were incorporated into each revision since publication of Revision 1.0 in September 2004.

SAM Revisions Tracking Table		
SAM Revision	**Publication Date**	**Changes incorporated summary**
Revision 1.0 EPA/600/R-04/126	September 2004	*Standardized Analytical Methods for Use During Homeland Security Events (SAM) 1.0* Included chemical and biological contaminants
Revision 2.0 EPA/600/R-04/126B	September 2005	• Added radiochemical contaminants • Added several persistent chemical warfare agent (CWA) degradation products • Added separate drinking water sample type for chemical and radiochemical contaminants • Added viability determination methods for pathogens • Added separate section for biotoxins
Revision 3.0 EPA/600/R-07/015	February 2007	• Added explosive chemicals • Combined identification and viability methods for pathogens • Added drinking water sample type for pathogens • Title changed to: *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM) 3.0*
Revision 3.1 EPA/600/R-07/136	November 2007	Developed a SAM website, to provide the SAM document and a format for searching and linking to SAM methods by analyte and sample type. (See https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.)
Revision 4.0 EPA/600/R-04/126D	September 2008	• Added wipe samples for chemical analytes • Added PCR methods for pathogens
Revision 5.0 EPA/600/R-04/126E	September 2009	• Added separate drinking water sample type to biotoxins section
2010 (Revision 6.0) EPA/600/R-10/122	October 2010	• Removed non-aqueous liquid sample type from chemical section • Temporary removal of pathogens

SAM Revisions Tracking Table		
SAM Revision	**Publication Date**	**Changes incorporated summary**
2012 (Revision 7.0) EPA/600/R-12/555	July 2012	• Changed title to: *Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2012* • Added vegetation sample type, newly available rapid methods, and total activity screening procedure for radiochemical analytes • Re-introduced pathogen methods with restructuring to clarify method applications for site characterization and post remediation • Assigned applicability tiers to chemical methods
2017 (Revision 8.0) EPA/600/R-17/356	October 2017	• Added outdoor building and infrastructure material sample types for radiochemical analytes • Added soil sample type for pathogens • Assigned applicability tiers to pathogen and biotoxin methods • Added analytes to chemical, radiochemical, pathogen and biotoxin sections • Added considerations regarding the potential impacts of decontamination agents on the analytical performance of selected radiochemical methods • Changed the names of "aqueous liquid" (chemical methods sample type) and "liquid water" (biotoxin methods sample type) to "non-drinking water" to clarify that the sample type applies to all non-drinking water aqueous sample matrices
2022 (Revision 9.0) EPA/600/R-21/320	September 2022	• Added analytes to chemical, radiochemical and biotoxin sections • Changed the name of the "aerosol" sample type to "air" for pathogens • Changed the name of the "particulate" sample type to "surfaces" for pathogens • Combined drinking water and post-decontamination wastewater into a single sample type for pathogens • Added limestone as a sample type for radiochemicals in outdoor infrastructure and building materials

The following documents and tools have been developed by EPA to provide information regarding a contamination incident. The information included in these documents is intended to be complementary to information provided in the analytical methods that are listed in SAM. As additional documents containing similar complementary information become available, they will be added to the list contained in this Attachment.

- Searchable Tools on the SAM webpage at: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam.

- The Sample Collection Information Documents provide information regarding sample containers/media, preservation, holding time, sizes, packaging and shipping, pertaining to collection of samples to be analyzed for the chemical, radiochemical and biotoxin analytes. The latest Sample Collection Information Documents are available at: https://www.epa.gov/esam/sample-collection-information-documents-scids

- U.S. EPA. 2009. "Guide for Development of Sample Collection Plans for Radiochemical Analytes in Environmental Matrices Following Homeland Security Events." Cincinnati, OH: U.S. EPA. EPA/600/R-08/128.

  This document provides a framework to assist incident commanders, project managers, state and local authorities, contractors and enforcement divisions in developing and implementing an approach for sample collection during the cleanup of an urban environment after a radiological contamination incident. Information in this document can be used to develop a systematic and integrated methodology for sample collection to meet data use needs and site disposition objectives. https://www.epa.gov/sites/production/files/2015-07/documents/guide_for_developing_sample_collection_plans_for_radiochemical_analytes.pdf

- U.S. EPA. 2010. "Rapid Screening and Preliminary Identification Techniques and Methods – Companion to SAM Revision 5.0." Cincinnati, OH: U.S. EPA. EPA/600/R-10/090.

  This document provides information regarding procedures for use when multiple laboratories are needed to perform rapid preliminary analysis of environmental samples following a contamination incident. The information is intended to support the analytical methods listed for chemical and radiochemical analytes in SAM Revision 5.0. https://www.epa.gov/sites/production/files/2015-07/documents/rapid_screening_and_preid.pdf

- U.S. EPA. 2016. "Sample Collection Procedures for Radiochemistry Analytes in Outdoor Building and Infrastructure Materials." Cincinnati, OH: U.S. EPA. EPA/600/R-16/128.

  This document provides instructions regarding the collection of samples from outdoor building and infrastructure materials to be analyzed for radiological contaminants following a contamination incident. The document focuses on the Site Characterization, Remediation and Final Status Survey (site release) phases of an incident and is not intended to address sample collection needs during Initial Response. The procedures are intended for collection of samples to be analyzed using the methods in SAM 2017. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=335065

- U.S. EPA. 2019. "Laboratory Analytical Waste Management and Disposal Information Document Companion to Selected Analytical Methods for Environmental Remediation and Recovery." Cincinnati, OH: U.S. EPA. EPA/600/R-90/116.

  This document addresses laboratory disposal of samples and associated analytical waste unique to remediation activities following a contamination incident, and assumes specific environmental sample types (i.e., water, soil, particulates and air collection media) to be analyzed using the methods listed in SAM. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=348313&Lab=CESER

- U.S. EPA. 2020. "Guide for Development of Sample Collection Plans for Radiochemical Analytes in Outdoor Building and Infrastructure Materials Following Homeland Security Events." Cincinnati, OH: U.S. EPA. EPA/600/R-20/097.

  This document provides a framework to assist incident commanders, project managers, state and local authorities, contractors and enforcement divisions in developing and implementing an approach for sample collection during the cleanup of outdoor buildings and infrastructure after a radiological contamination incident. Information in this document can be used to develop a systematic and integrated methodology for sample collection to meet data use needs and site disposition objectives. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=349143&Lab=CESER

- U.S. EPA. 2020. "Sample Collection Procedures for Radiochemistry Analytes in Environmental Matrices." Cincinnati, OH: U.S. EPA. EPA/600/R-20/247.

  This document focuses on the Site Characterization, Remediation, and Final Status Survey (site release) phases of a contamination incident and is not intended to address sample collection needs during Initial Response. The procedures are intended for collection of environmental samples in response to a radiological contamination incident at the point where Federal Radiological Monitoring and Assessment Center (FRMAC) activities are turned over to EPA. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=350579&Lab=CESER

- U.S. EPA and U.S. Geological Survey. 2014. USEPA/USGS Sample Collection Protocol for Bacterial Pathogens in Surface Soil. U.S. Environmental Protection Agency: Cincinnati, OH and U.S. Geological Survey, St. Petersburg, FL, EPA/600/R-14/027.

  This sample collection procedure describes activities and considerations for collection of bacterial pathogens from surface soil samples at depths (0-5 cm) that can be reached without the use of a drill rig, direct-push technology, or other mechanized equipment. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=285571

- Lee, S., W. Calfee, J. Archer, T. Boe, L. Mickelsen and D. Hamilton. 2017. "Field Application of Emerging Composite Sampling Methods." U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-17/212.

  The study discussed in this report tested the effectiveness of aggressive air sampling, robotic floor cleaner, and wet vacuum composite methods for sampling spores from a subway platform and rail surfaces. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=337466

- Silvestri, E., Y. Chambers-Velarde, J. Chandler, J. Cuddeback, K. Jones and K. Hall. 2018. "Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field." U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-18/164.

  This document summarizes elements that should be considered when planning, developing and implementing a sampling and analysis plan for microbiological contamination incidents. It is intended to be an informational companion to EPA's "Sampling and Analysis Plan (SAP) Template Tool for Addressing Environmental Contamination by Pathogens." https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=341832

- Silvestri, E., Y. Chambers-Velarde, J. Chandler, J. Cuddeback, J. Archer, and W. Calfee. 2021. "Collection of Microbiological Agent Samples from Potentially Contaminated Porous Surfaces Using Microvacuum Techniques." U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, EPA/600/R-20/439.

  This document provides step-by-step instructions for the use of vacuum filter cassettes to collect samples from surfaces potentially contaminated with pathogens. It is intended to be used in conjunction with the analytical methods listed in U.S. Environmental Protection Agency's Selected Analytical Methods for Environmental Remediation and Recovery (SAM) and in the Environmental Sampling and Analysis Method Program online query tools for SAM, following homeland security-related contamination incidents. The instructions are applicable to collection of *Bacillus anthracis* spores from surfaces using a 37-mm filter cassette and microvacuuming techniques, with either a mixed cellulose ester (MCE) filter or a polytetrafluoroethylene (PTFE) filter. Although testing has not been completed and collection efficiencies are unknown, these instructions might also be applicable to other pathogens.
  https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=352037&Lab=CESER

- Silvestri, E., Y. Chambers-Velarde, J. Chandler, J. Cuddeback, W. Calfee, J. Archer and S. Shah. 2021. "Collection of Surface Samples Potentially Contaminated with Microbiological Agents Using Swabs, Sponge Sticks and Wipes." U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, EPA/600/R-21/051.

  This document provides step-by-step instructions for the use of swabs, wipes, and sponge-sticks to collect samples from surfaces potentially contaminated with microbiological agents. It is intended to be used in conjunction with the analytical methods listed in U.S. Environmental Protection Agency's Selected Analytical Methods for Environmental Remediation and Recovery (SAM) and in the Environmental Sampling and Analysis Method Program online query tools for SAM, following homeland security-related contamination incidents.
  https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=352038&Lab=CESER

- Silvestri, E., Y. Chambers-Velarde, J. Chandler, J. Cuddeback, W. Calfee and J. Archer. 2021. "Collection of Air Samples Potentially Contaminated with Microbiological Agents Using Impingers, Impactors and Low-Volume Filters." U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, EPA/600/R-21-007.

  This document provides step-by-step instructions for the use of impingers, impacters and filters to collect samples from air potentially contaminated with pathogens. It is intended to be used in conjunction with the analytical methods listed in U.S. Environmental Protection Agency's Selected Analytical Methods for Environmental Remediation and Recovery (SAM) and in the Environmental Sampling and Analysis Method Program online query tools for SAM, following homeland security-related contamination incidents.
  https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=352040&Lab=CESER

- Silvestri, E., J. Cuddeback, K. Hall, T. Haxton, C. Jones, And J. Falik. 2021. "Sampling and Analysis Plan (SAP) Template Tool for Addressing Environmental Contamination by Pathogens" and corresponding User's Guide. U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, EPA/600/R-21/144.

  The User Guide and corresponding Template Tool are provided to facilitate generation of an outline that can be used to develop sampling and analysis plans (SAPs) in support of exercises, research studies or remediation activities following a contamination incident involving pathogens in environmental matrices. The guide and template are applicable for phases of a contamination incident in which EPA is responsible for conducting sampling and analysis activities, and provide a general description of the types of information and sections that would be included in a SAP for sampling and analysis activities associated with environmental matrices potentially containing pathogens. The

fillable Template Tool is meant to be used as a "ready-to-go" outline for creating a SAP in EPA-report format. The template also facilitates capturing information associated with the data quality objective (DQO) process, including generation of a DQO summary.
https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=353154&Lab=CESER